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Acute, Five- and Ten-Day Inhalation Study of Hydroprocessed Esters and Fatty Acids - Mixed Fats (HEFA-F) Jet Fuel

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Air Force Research Laboratory
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The experiments reported were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

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14. ABSTRACT
The U.S. Air Force is pursuing development of alternative fuels to augment JP-8 jet fuel. Hydroprocessed Esters and Fatty Acids from Mixed Fats (HEFA-F) jet fuel was administered as an aerosol-vapor mixture to 5 male and 5 female Fischer-344 rats/group. Inhalation exposures lasted 6 hours/day for 1 day (with and without an 11-day recovery), 5 days or 10 days (5 days/week, 2 weeks). Exposure concentrations were 0, 200, 700 and 2000 mg/m^3; mean aerosol measurements were 0, 7, 22 and 28 percent, respectively. There were no significant changes in body weights at any time point. Urinalysis changes included a slight pH decrease in all exposed rats; a small elevation in ketones and leukocytes and hemoglobin were present in the 2000 mg/m^3 males. There were no changes in standard clinical chemistry or hematology parameters. Caudal lung tissue was analyzed for cytokines, chemokines and receptors; no significant changes were seen. Proinflammatory blood cytokines showed no significant differences. Male kidney weight increases were likely related to male rat specific hyaline droplet formation. Nasal cavity changes included olfactory epithelial degeneration at 2000 mg/m^3. Alveolus inflammation was seen in the 2 higher doses. To examine sensory irritation, male Swiss-Webster mice were exposed nose-only to 1916 mg/m^3 HEFA-F for 30 minutes, resulting in 23 percent respiratory depression.

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PREFACE

Funding for this project was provided through the Air Force Research Laboratory, Propulsion Directorate, Fuels Branch (Dr Tim Edwards, AFRL/RZPF). This research was conducted under contract FA8650-10-2-6062 with the Henry M. Jackson Foundation for the Advancement of Military Medicine. The program manager for the contract was David R. Mattie, PhD, who was also the technical manager for this project. The authors acknowledge John Hinz (USAFSAM/OEHR) for assistance in designing portions of the study.

The study protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (U.S. EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870. 1300 Acute Inhalation Toxicity (1998) and the Organization for Economic Co-operation and Development Guideline OECD 412 Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (OECD, 1981).

This animal study was approved by the Air Force Surgeon General's Human and Animal Research Panel (protocol number FWR-2010-0003A) and the Hamner Institutes for Health Sciences Animal Care and Use Committee (protocol number 100006). The study was conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

1.0 SUMMARY

The U.S. Air Force is pursuing the development of alternative fuels to augment or replace petroleum-based JP-8 jet fuel. One class of synthetic alternative jet fuel is manufactured using a Hydro-treated Renewable Jet (HRJ) manufacturing process from which the resulting fuel is called Hydroprocessed Esters and Fatty Acids (HEFA). The fuel studied herein is manufactured from mixed animal fats and oils and is referred to as Hydroprocessed Esters and Fatty Acids-Mixed Fats (HEFA-F).

The study was designed to assess HEFA-F administered as an aerosol and vapor mixture by inhalation exposure to rats for six hours per day for one day (acute) with and without a recovery period, a five day duration and a ten day duration (five days per week for two weeks with a two day recovery in between). At each of these time points, both male and female rats were exposed to 0, 200, 700 or 2000 mg/m^3 HEFA-F. An assessment of sensory irritation potential was also conducted using male Swiss-Webster mice.

There were no clinical observations or body weight changes attributed to HEFA-F exposure. Observed decreases in food and water consumption in HEFA-F exposed rats were not consistently significant compared to control groups and were considered random due to small group sizes. In urine samples collected from animals of the ten-day duration groups, pH appeared slightly lower in HEFA-F-exposed animals; leukocytes were present with slightly elevated ketones and hemoglobin in the 2000 mg/m^3 male rats. There were no HEFA-F exposure-related differences in blood chemistry endpoints in female rats. Observed blood chemistry changes in HEFA-F exposed male rats were not concentration-related; these effects were not considered to be associated with HEFA-F toxicity.

At necropsy, no gross lesions were attributed to HEFA-F exposure. In male rats, increases in kidney weights were consistent with rat-specific hydrocarbon induced hyaline droplet formation. Hyaline droplets were observed in the kidneys of male rats exposed to 700 and 2000 mg/m^3 HEFA-F concentrations in either the five- or ten-day duration groups. Nasal cavity changes induced by HEFA-F were characterized by dose-dependent olfactory epithelial degeneration with an average degree of severity of slight/mild. Increase of exposure duration (acute to five- to ten-day) did not change the observed degree of severity. Squamous, transitional or respiratory epithelium inflammation was observed in the anterior region of the nasal cavity of both HEFA-F exposed and control animals. Focal chronic inflammation was also observed in the lungs of both HEFA-F exposed and control rats. Dose-dependent incidence of alveolus inflammation was observed in HEFA-F exposed rats, regardless of duration of exposure (acute, five- or ten-day). There were no HEFA-F exposure-related histopathological changes observed in the larynx, trachea, liver, spleen, heart or adrenals.

No significant changes were seen in right caudal lung tissue analyzed for cytokines, chemokines and receptors using PCR arrays. Analysis of proinflammatory cytokines in blood did not find any significant changes.

A combined vapor/aerosol exposure of 1916 mg/m^3 HEFA-F evoked a slight to moderate sensory irritation in mice, with a group mean decrease in respiratory rate of 23 percent.

2.0 INTRODUCTION

2.1 Background

The U.S. Air Force is pursuing the development of alternative fuels to augment or replace petroleum-based JP-8 jet fuel. The use of alternative fuels can help decrease the dependence on foreign oil sources. Alternative fuels may be developed by synthesis from simpler molecules (e.g., natural gas) or by refining from non-petroleum sources (e.g., biologically-based sources). The Air Force Research Laboratory/Molecular Bioeffects Branch (711 HPW/RHDJ) has previously conducted studies on the inhalation toxicity of a synthetic jet fuel produced using a Fischer-Tropsch process from natural gas, now referred to as Synthetic Paraffinic Kerosene (SPK). These studies looked at the toxicity of acute, ten-day and 90-day exposure durations via inhalation to multiple concentrations of SPK fuel up to 2000 mg/m^3 and sensory irritation produced by SPK (Hinz *et al.*, 2012; Mattie *et al.*, 2011a, 2011b). These studies provided the framework for the design of the toxicity study in this report.

A second class of synthetic alternative jet fuel is manufactured using a Hydro-treated Renewable Jet (HRJ) manufacturing process from which the resulting fuel is called Hydroprocessed Esters and Fatty Acids (HEFA). The HEFA biofuels are generated primarily from natural oils and fats. The three HEFA biofuels being examined by this laboratory are from camelina oil (camelina plant seeds), tallow (rendered animal fat) and from mixed animal fats and oils. The fuel studied herein is manufactured from mixed fats and oils and was initially called HEFA-F for renewable JP-8, but will be referred to in this report as Hydroprocessed Esters and Fatty Acids-Mixed Fats (HEFA-F).

Regardless of the fuel source, toxicity testing is required to ensure the safety of personnel working with jet fuel. The greatest opportunity for exposure to a jet fuel is during refueling operations. At these times, personnel may be exposed to vapors and aerosols of jet fuel produced by the refueling process; exposure is primarily by dermal contact or inhalation. Since inhalation is one of the two major routes of exposure for JP-8, the assessment of toxicity of HEFA-F fuel by inhalation is needed to assess the risk of replacing or augmenting JP-8 with this renewable fuel.

One objective of this study was to determine the inhalation toxicity of HEFA-F, in order to compare results with baseline inhalation studies for JP-8 and SPK. Another objective was to compare acute and short-term exposures to determine if they might be sufficient for assessing the inhalation hazard of alternative jet fuels. The overall study was designed to assess HEFA-F administered as an aerosol and vapor mixture by inhalation exposure to rats for six hours per day for one day (acute) with and without a recovery period, a five day duration, and a ten day duration (five days per week for two weeks with a two day recovery in between). At each of these time points, both male and female rats were exposed to 0, 200, 700 or 2000 mg/m^3 HEFA-F. The assessment included clinical observations, urinalysis, clinical pathology, gross pathology, histopathology and lung cytokine/chemokine screening for the two week study. The shorter time points were only assessed by histopathology, clinical observations, body weights and organ weights.

The acute duration protocol was designed to be in general compliance with the U.S. Environmental Protection Agency (U.S. EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870. 1300 Acute Inhalation Toxicity (1998). The ten-day duration exposure followed the guidelines of the Organization for Economic Cooperation and Development Guideline (OECD) 412 Repeated Dose Inhalation Toxicity: 28-Day or 14-Day study (OECD, 1981). The acute (six-hour with eleven-day recovery) and the five-day duration exposures were designed to enhance the investigation of short term effects from HEFA-F. An assessment of sensory irritation potential was also conducted using mice. This assay followed, in part, the ASTM International standard E-981-04 (2004) which was derived from studies by Alarie (1966, 1973), who established the dose response relationship between breathing rate depression and occupational standards.

3.0 METHODS

3.1 Experimental Design

These exposures were designed to assess the potential inhalation toxicity of the alternative jet fuel HEFA-F when administered as an aerosol and vapor combination via whole-body inhalation. Rats were exposed to HEFA-F for one of three durations: once for six hours per day (acute), six hours per day for five consecutive days, or six hours per day for ten days (five consecutive days with a two-day break followed by five more consecutive days) at concentrations of 0, 200, 700 and 2000 mg/m^3. Five male and five female Fischer-344 rats were used (Table 1) for each exposure group. Animals were euthanized and necropsied after the completion of exposures and/or observation/recovery periods. Assessments included body weights, clinical observations, food and water consumption, urinalysis and clinical chemistries, gross pathology and histopathology of target organs.

Additionally, four male Swiss-Webster mice were exposed to HEFA-F as a single high concentration aerosol and vapor combination via nose-only inhalation. This exposure assessed the sensory irritation potential of HEFA-F.

3.2 Test Substance

The alternative jet fuel HEFA-F was obtained from the Syntroleum Corporation (Tulsa OK) by the Fuels Branch at Wright-Patterson Air Force Base (AFRL/RQPF), where it was identified as POSF 5469. The fuel was manufactured as HEFA-F Renewable Jet Fuel, CAS Number 437986-20-4. Chemical and physical properties of this fuel can be found in Appendix A. The jet fuel was stored in a well-ventilated area under room temperature ambient conditions.

Table 1. Rat Exposure Group Design

Exposure Target Concentration (mg/m^3)	Exposure Duration							
	Acute		Acute + Recovery		Five-Day		Ten-Day	
	Number of F-344 Rats							
	M	F	M	F	M	F	M	F
0 (Control)	5	5	5	5	5	5	5	5
200	5	5	5	5	5	5	5	5
700	5	5	5	5	5	5	5	5
2000	5	5	5	5	5	5	5	5
(Total)	20	20	20	20	20	2	20	20

Notes: F = females; M = males

3.3 Test Animals

A total of 85 male and 85 female rats (Fischer (CDF®) [F344/DuCrl]) were obtained from Charles River Laboratories (Kingston NY). Additionally, six male mice (Swiss-Webster CRL:CFW(SW)) were obtained from the same supplier. All animals were examined by the animal care staff; 80 male rats, 80 female rats and four male mice were selected on the basis of physical examination and body weight and considered suitable for the study. Animals were acclimated to the facility for approximately two weeks. During the acclimation period, rats were individually housed in stainless steel wire-mesh cages (R-24 cage units, Lab Products, Inc., Seaford DE) and mice were group-housed (two to three animals) in micro-isolator-type housing. Room conditions were maintained at a target of 22 °C, 30-70 percent humidity, with a 12 hour light/dark cycle.

Animals were fed a certified rodent diet, NIH-07 pellets (Zeigler Brothers, Gardners PA) and were provided reverse osmosis purified municipal tap water, *ad libitum*, except during exposure, when food was withheld. Certification of analysis of feed batch was supplied by the manufacturer. There were no known contaminants in the feed that were expected to interfere with the results of this study. Drinking water analyses were conducted quarterly by an independent laboratory. There were no known contaminants in the drinking water that were expected to interfere with the results of this study.

Prior to exposure, rats were weighed and assigned randomized animal numbers using an Instem Provantis 8™ protocol (Provantis, Conshohocken PA). At least three days prior to start of exposure, animals in wire mesh caging were transferred to the 1 m^3 chambers (H1000, Lab Products, Seaford DE) for housing acclimation.

Mice were weighed and those in the appropriate weight range were selected for the assay. No randomization of mice was needed as there was only one experimental group to assess the potential for sensory irritation. To reduce stress during exposure, mice were acclimated to

modified nose-only plethysmograph tubes (Model 3381, Buxco Research Systems, Wilmington NC) for one hour on the day preceding the assay.

3.4 Test Substance Administration

The test substance was administered to the rats by whole-body inhalation and to mice by nose-only inhalation. During whole-body exposures, the cage racks containing rats were transferred into the 1 m^3 exposure chambers and were exposed to the aerosol-vapor mixture of jet fuel for six hours per day for one-, five- or ten- (five days with a two-day break followed by five more days) day durations. After each day's exposures, animals were transferred back to separate 1 m^3 chambers for housing during non-exposure periods. Food and water consumption were measured for the ten-day duration group during the two-day break.

Air for the exposure chambers was pulled by fans through a 95 percent high efficiency particulate air (HEPA) filter and a charcoal filter. Temperature and humidity were adjusted as required and the air was distributed to the exposure chambers. Air flow was measured by monitoring the pressure drop across an orifice plate at the inlet to each chamber. Air flow was calibrated using an in-line mass flow meter (Sierra Instruments, Inc., Monterey CA). The temperature and relative humidity in the chambers was measured by using a humidity temperature transmitter (Hygromer 200 Series, Rotronic AG, Huntington NY) located near the center top of the chamber. The temperature transmitter was calibrated by comparison with a certified thermometer and humidity was calibrated by comparison with saturated salt solutions.

3.4.1 Frequency and Duration of Administration: HEFA-F was administered to rats for approximately six hours per exposure day. Rats were exposed for one day, five consecutive days, or ten days (five consecutive with a two-day break followed by five consecutive days).

3.4.2 Generation of Test Material: HEFA-F was administered as a mixture of aerosol and vapor in the breathing air of the animals. The test atmosphere was generated by pumping the liquid jet fuel into an air atomizing nozzle (Model SUJ1A with fluid cap 1650 and air cap 64, Spraying Systems Co., Wheaton IL). A liquid metering pump (FMI, Fluid Metering, Inc., Syosset NY) transferred liquid jet fuel from a glass bottle reservoir to the nozzle (Figure 1). Compressed instrument air at approximately 50 psi was supplied to the nozzle. The nozzle assembly was housed in a stainless steel sanitary tee fitting. The spray was directed into a custom-made glass mixing vessel of approximately 13 L (Figure 2). The total flow of the chamber passed through the glass tube, carrying the generated jet fuel mixture into the exposure chamber (Figure 3).

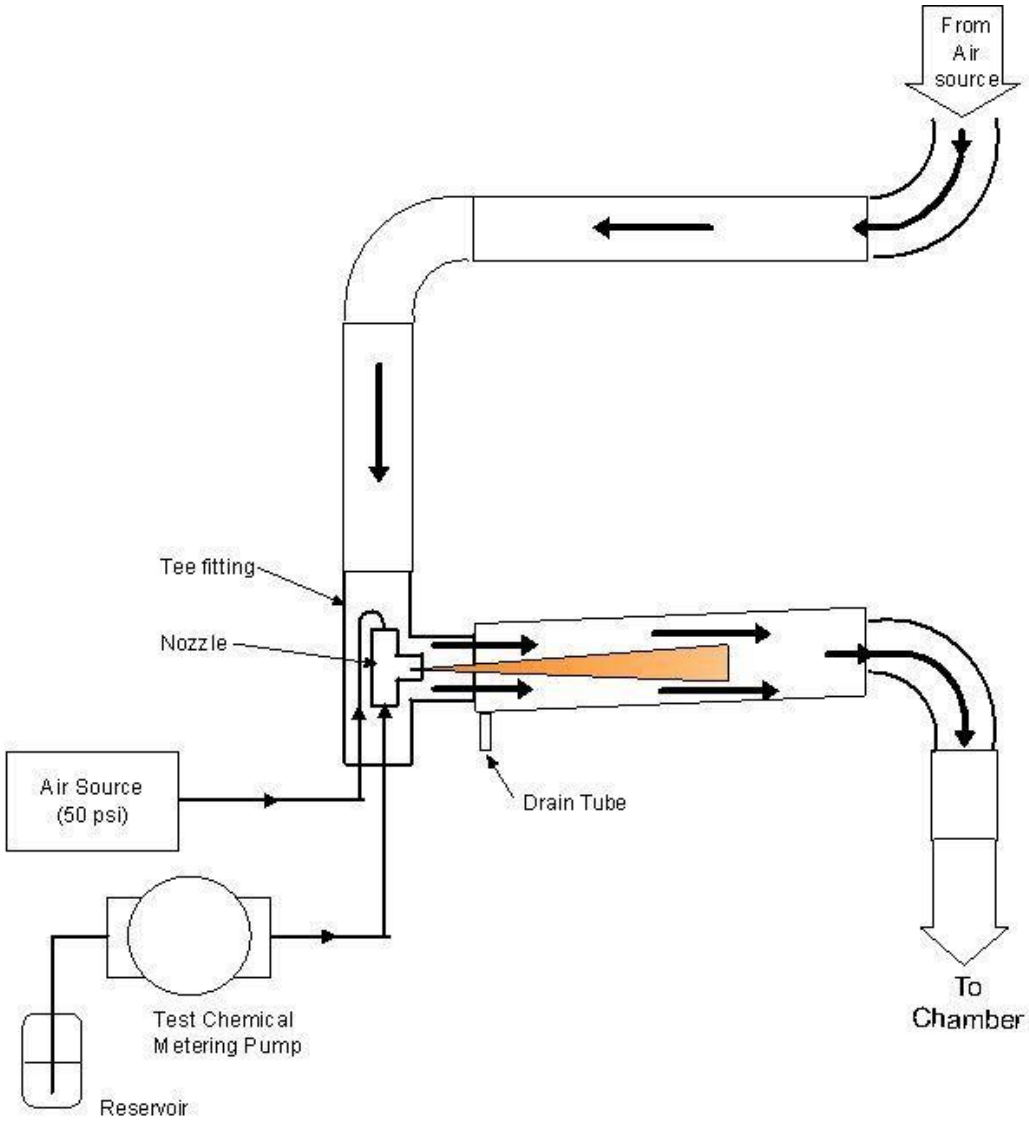


Figure 1. Diagram of HEFA-F Jet Fuel generation system showing the spray nozzle, glass mixing tube and associated parts

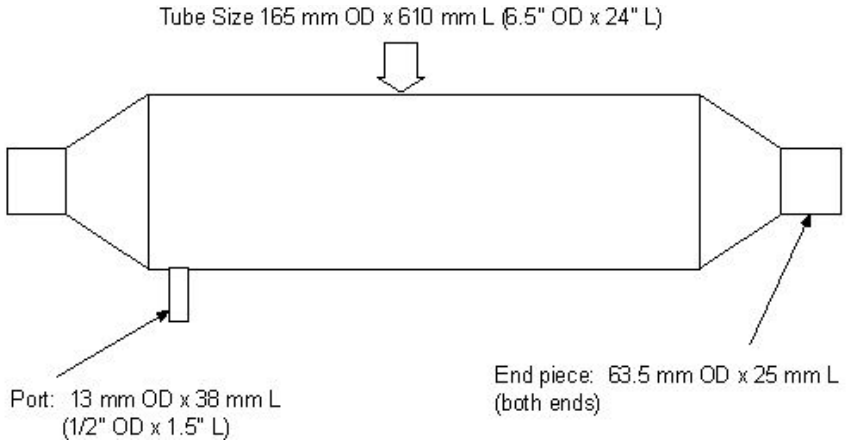


Figure 2. Custom-made glass mixing tube for generation system. Overall length is 834 mm.

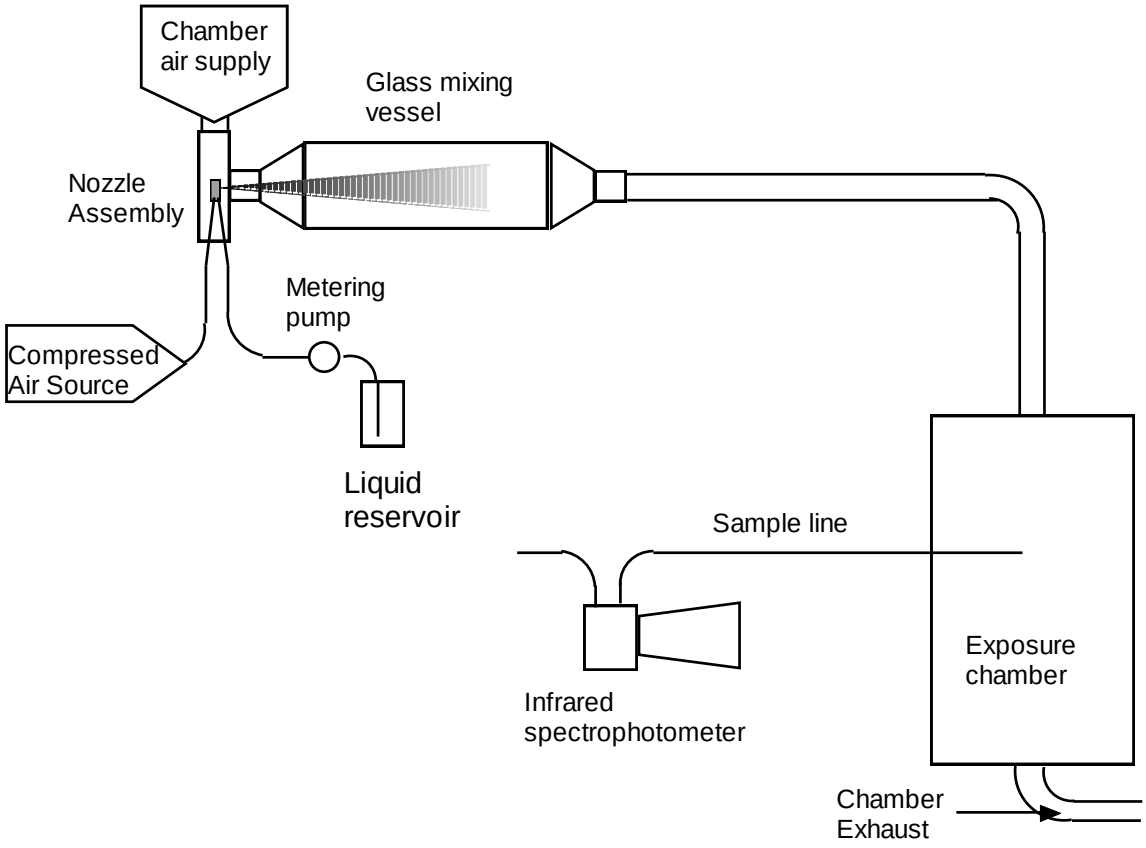


Figure 3. Jet Fuel Generation and Exposure System Schematic

3.4.3 Monitoring of Test Material: An infrared spectrophotometer (MIRAN 1A, Foxboro Co., South Norwalk CT) was used to monitor the concentration of jet fuel in the chamber. The

sensing cell of the infrared (IR) spectrophotometer was warmed to approximately 50 °C by heat tape. Operating conditions for the spectrophotometer can be found in Appendix A. A sample of the chamber atmosphere was pulled through the IR spectrophotometer. As the sample moved through the heated cell, the aerosol droplets evaporated. A chart recorder was used to continuously record the electrical output of the IR spectrophotometer.

The IR spectrophotometer was calibrated using a closed loop method. Jet fuel was injected in a series of volumes to produce a set of increasing concentrations of jet fuel. A calibration curve of spectrophotometer response as a function of jet fuel concentration was produced.

Nominal concentration was calculated from the air flow rate through the chamber and the mass of the material used each exposure day.

3.4.4 Summary of Chamber Activity: The uniformity of distribution within the exposure chamber was checked by measuring the concentration at nine different locations within the chamber. Chamber distribution measurements were conducted using the infrared spectrophotometer and it was determined that the variability in chamber concentration was 6.1 percent or less, indicating that the distribution of the test compound within the chamber was uniform.

Aerosol concentration was determined by taking a gravimetric filter sample from the front of each chamber door. A sample of the atmosphere was pulled through the filter at a known flow rate and time. The aerosol concentration was calculated from the mass of the jet fuel collected on the filter and the volume of atmosphere pulled through the filter.

Particle size distribution measurement was conducted using an aerodynamic particle sizer (APS, Model 3321, TSI, Inc., St. Paul MN). The instrument was connected to a sample port on the chamber. Dilution air was added in order to prevent the aerosol concentration from reaching overload conditions. Chamber data can be found in Appendix A.

3.5 Sensory Irritation Potential

HEFA-F was administered to mice by nose-only inhalation for a single 30 minute time period according to ASTM standard E-981-04 (2004). Four male mice were loaded into nose-only plethysmographs, which isolated the animal head from its body via a latex dam. The plethysmographs were placed onto the nose-only exposure tower (Jaeger-NYU, CH Technologies Inc, Westwood NJ).

Dilution air for exposures was pulled by fans at approximately 225 L/min through a 95 percent HEPA filter and a charcoal filter, the temperature and humidity adjusted, as required, and distributed to the exposure system. The exposure atmosphere was generated, mixed with the dilution air and delivered into a 1 m^3 steel and glass inhalation chamber. The inhalation chamber was used to further mix exposure atmospheres. Atmospheres were then drawn from the

inhalation chamber to the nose-only exposure tower at approximately 2 L/min (Figure 4). The mixed vapor/aerosol atmospheres were generated as described in Section 3.4.2.

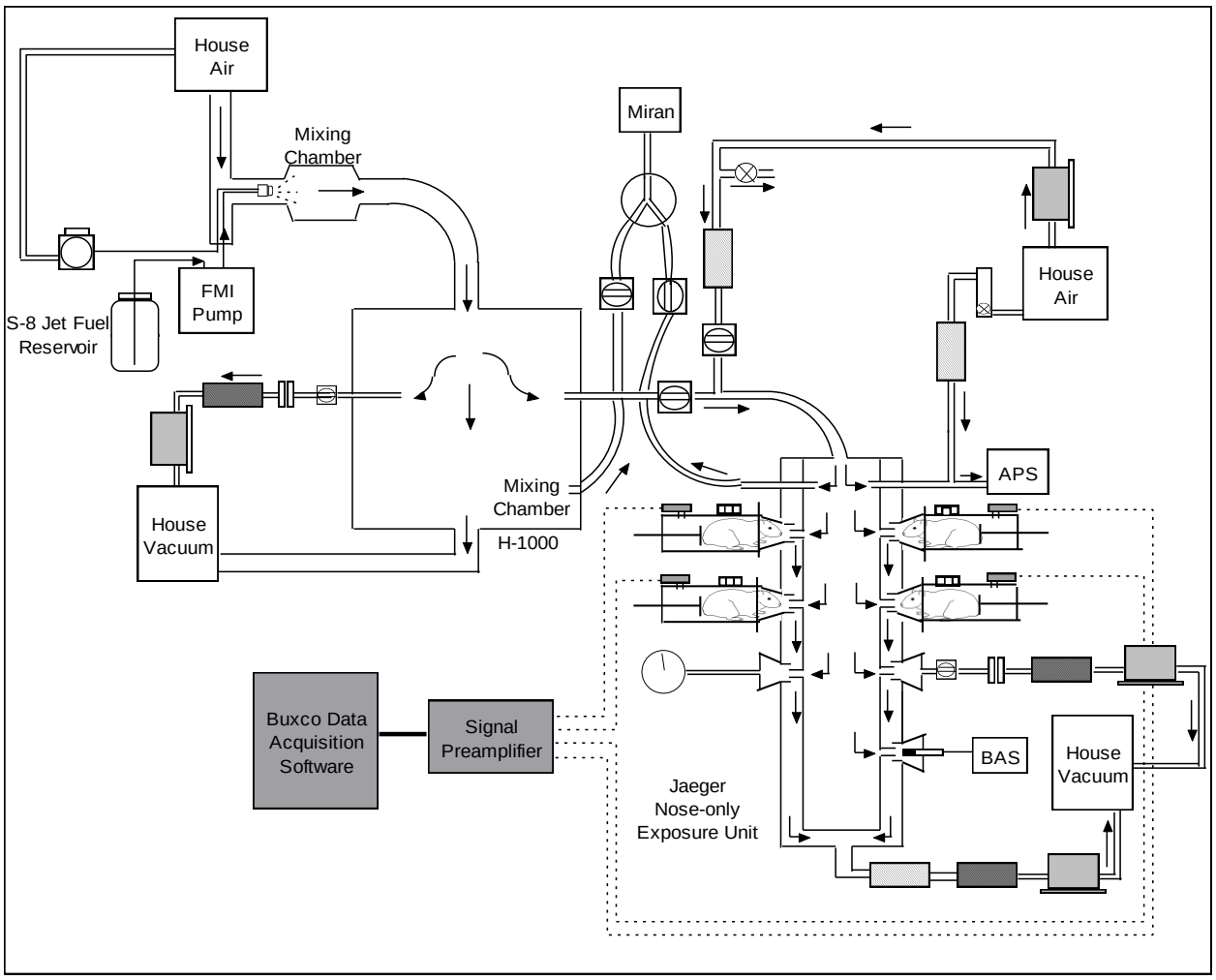


Figure 4. Sensory Irritation Assessment Atmosphere Generation and Exposure System

Monitoring of test material was performed as previously described (Section 3.4.3). A mass weight (gravimetric) filter (MWF) sample was used to determine the concentration of the non-volatile aerosol. A filter was collected from a port on the nose-only exposure unit for the exposure. Particle size distribution measurement was conducted using an APS connected to the inside of the nose-only exposure unit. Dilution air was added in order to keep the aerosol concentration out of overload conditions. APS sampling was conducted prior to, during and after the exposure.

Temperature and relative humidity were measured at the nose-only tower by a Rotronic Humidity Sensor (Series 200, Rotronic Instrument Corp., Huntington NY) connected to the Continuum Building Automation System (Andover Controls Corporation, Andover MA). Mean

temperature and humidity (± standard deviation (SD)) were 69.9 (± 0) °F and 45.3 (± 5) percent relative humidity, respectively.

While breathing clean air, a baseline measurement of respiratory frequency was recorded for ten minutes. Measurement of respiratory frequency was continued as mice were exposed to HEFA-F (2000 mg/m^3) for 30 minutes. After 30 minutes of exposure, the mice were exposed to clean air for ten minutes and respiratory frequency was measured and compared to initial baseline readings.

Sensory irritation was measured by recording animal breathing signals. As the animal's body expanded and contracted during normal breathing, the pressure in the plethysmograph fluctuated accordingly. Pressure fluctuations forced air to flow through an opening at the top of the plethysmograph. These airflows passed through a stainless steel mesh pneumotachograph. The pressure difference across the pneumotachograph was detected by a pressure transducer, which converted the pressure differential to an electrical signal. The electrical signal captured by the data acquisition system was in the form of the animals breathing pattern. The breathing patterns were amplified, digitally recorded and analyzed for respiratory rates by the Buxco Biosystem XA software (Version 2.7.9, Buxco Research Systems, Wilmington NC). The average baseline respiratory rate, the lowest representative respiratory rate during the exposure and the highest post-exposure rate were determined for each animal in the group.

The baseline respiratory rates were averaged over the last six 15-second intervals immediately prior to the exposure period. Exposure respiratory rates were averaged at 15-second intervals for the first five minutes and at three-minute intervals for the remainder of the exposure period. Post-exposure breathing rates were averaged at one-minute intervals. The lowest representative breathing rate and the highest post-exposure rates were each divided by the baseline rate to obtain a "percent of baseline" value. The percent of baseline value was subtracted from 100 percent to yield the response by each animal (percent decrease in respiratory rate).

Following the end of the 30 minute assessment, the mice were euthanized by carbon dioxide inhalation followed by exsanguination.

3.6 Experimental Evaluations

3.6.1 Viability Checks and Clinical Observations: Rats and mice were observed for morbidity, mortality, general appearance and signs of severe toxic or pharmacological effects before and after exposure, and at least once daily during non-exposure periods. Detailed examinations for clinical signs of disease or abnormality were performed prior to the start of exposures and at each subsequent weighing. These examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, as well as evaluations for respiration, circulatory effects, autonomic effects, central nervous system effects and reactivity to handling or sensory stimuli. Lack of clinical signs during these examinations was recorded as no abnormalities detected (NAD).

3.6.2 Body Weights: Each rat was weighed within two days after arrival, at randomization, twice weekly and at necropsy. Mice were weighed within two days after arrival and within two days prior to the sensory irritation assessment.

3.6.3 Food and Water Consumption: Food and water consumption was measured during the two-day break between exposure weeks one and two for rats in the ten-day exposure group. Following the exposure on the fifth day of week one, animals in the ten-day exposure group were transferred to individual polycarbonate caging. Food pellets were added to the stainless steel wire cage lid hopper and weighed. Water bottles containing reverse osmosis water were weighed and attached to caging. On the first morning following the two-day break and prior to start of exposure, rats were transferred back to the exposure chambers. The stainless steel wire cage lids containing food pellets and the water bottles were weighed to determine the amount of food and water consumed.

3.6.4 Necropsy and Histopathology: The morning following the final exposure, necropsies were performed on each rat. Necropsies were performed across exposure concentration groups to minimize bias. Scheduled rats were brought to the necropsy facility where a terminal body weight was collected and rats were deeply anesthetized with sodium pentobarbital. Blood for clinical chemistry was collected via cardiac puncture from rats in the ten-day exposure groups. All animals were exsanguinated by trans-section of the abdominal aorta; and then necropsied. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. After dissection, wet weights of adrenal glands, kidneys and liver were obtained from the exposed and control animals; tissues were then placed in neutral buffered formalin (NBF).

Prior to preserving the lung tissue, the right caudal lobe of the lung was removed and placed in RNA preservative for the cytokine screen.

Histology slides were prepared at Experimental Pathology Laboratories, Inc. (EPL, Research Triangle Park (RTP) NC) and microscopic examinations were performed there. Tissues were processed, embedded and 5 μm sections were prepared from each block. Sections were then stained with hematoxylin and eosin. These tissue sections included the nasal airways (six sections), trachea, larynx, lung (whole left lobe), liver (left and median lobe), kidney (right and left), spleen, adrenals (right and left), heart and any gross lesions. Histological tissue sections were evaluated via light microscopy by the board-certified pathologist.

Right cranial lobes of lungs from five treated and five control animals were stained with Gomori methenamine silver (GMS) and gram stains to screen for fungal and bacterial infections, respectively.

3.6.5 Urinalysis: Urine was collected from four male and four female rats for each exposure concentration in the ten-day duration groups. Rats were placed in metabolism cages the day

prior to terminal necropsy and urine was collected on ice overnight. Urine volume and water consumed were measured and recorded. Urine specific gravity was measured and urine was analyzed semi-quantitatively for leukocytes, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin and blood (hemoglobin).

3.6.6 Clinical Chemistry: Blood was collected from male and female rats in the ten-day exposure groups via cardiac puncture following pentobarbital anesthesia. Serum was harvested and sent to Antech GLP (Antech Diagnositics, RTP NC) for assessment of clinical chemistry endpoints.

3.6.7 Cytokine/Chemokine Screen: The right caudal lung tissue from all rats was analyzed for inflammatory cytokines and receptors. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad CA). The isolated RNA was further purified using RNeasy columns (Qiagen, Valencia CA) and the integrity of the RNA was verified spectrophotometrically with a Nanodrop Spectrophotometer (Thermo Scientific, Wilmington DE) and with the Agilent 2100 Bioanalyzer (Santa Clara CA). Double-stranded cDNA was synthesized from 1 µg of total RNA using the RT2 First Strand synthesis kit (SABiosciences, Frederick MD). Cytokine gene expression was determined from 500 ng of cDNA using an RT2 Profiler PCR Array for Rat Inflammatory Cytokines and Receptors Kit (SABiosciences) on an ABI 7900HT Sequence Detection System (ABI, Foster City CA).

3.7 Statistical Evaluation

Descriptive statistics (mean, standard deviation and sample size) were used to summarize the in-life data and were reported for body weight and body weight gain, food and water consumption and clinical chemistry. Tests of significance for in-life data were computed by an Instem Provantis 8^{TM} protocol (Provantis, Conshohocken PA). The level of statistical significance was accepted at $p<0.05$, as evaluated by the Williams test (Williams, 1971, 1977).

Lung cytokines PCR array data were analyzed using SABioscience's online RT2 Profiler PCR Array Data Analysis web site (<http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php>) utilizing the provider's recommended default settings and the Student's t-test to evaluate significance. Data were analyzed by dose response using pooled gender samples, as well as each gender separately. For dose-response, samples were pooled by exposure concentration groups:

Control group = all males and females, ten-day exposure, 0 mg/m^3 (40 samples total)
Group 1 = all males and females, ten-day exposure, 200 mg/m^3 (40 samples total)
Group 2 = all males and females, ten-day exposure, 700 mg/m^3 (40 samples total)
Group 3 = all males and females, ten-day exposure, 2000 mg/m^3 (40 samples total)

For gender response, samples were pooled across all time points as above, but male and female groups analyzed separately (20 samples per dose). In both cases, only Ct values less than 35 were used in computations (recommended cut-off value by SABiosciences).

4.0 RESULTS

4.1 Exposure Period

An exposure day was defined as a six-hour period from approximately 8:00 am until 2:00 pm. The exposure period began when compressed air and jet fuel began to flow into the nozzle. The concentration in the chamber began to increase immediately, as observed on the infrared spectrophotometer chart recording (data not shown). At the end of the exposure period, the compressed air and fuel flow to the nozzle were shut off. The aerosol concentration was observed to drop as expected. Each exposure was followed by a 30-minute clearance period for the low concentration and 60-minute period for the mid and high concentrations, to allow the chemical to significantly reduce in the exposure chamber prior to opening for animal care procedures. After the exposure and clearance period, cage units holding the animals were transferred to 1-m^3 chambers of the same model as the exposure chambers located in the same room. Control animals were exposed and housed within the same chamber.

4.2 Exposure Conditions

Over the course of the exposures, concentration, temperature, humidity, air flow and static pressure readings were recorded (Table 2). The average temperature, humidity and air flow remained at or near target set points and did not deviate outside of prescribed ranges, with the exception of three incidences of slightly high humidity readings lasting 30 minutes or less. These deviations in humidity did not affect the integrity of the exposures. The study average total concentrations, across time points, were 202 ± 8, 685 ± 23 and 1952 ± 72 mg/m^3 for the 200, 700 and 2000 mg/m^3 chambers, respectively. Nominal concentrations, based on the liquid pump flow rate and the chamber air flow, were 173.2 ± 8.2, 598.9 ± 44.6 and 2046.8 ± 76.8 mg/m^3, giving analytical to nominal concentration ratios of 1.16, 1.17 and 0.97, respectively.

Aerosol mass concentrations were measured using gravimetric filters. Filter samples were taken a minimum of three times a week over the course of the exposures. The average aerosol concentrations were 0.12 ± 0.11, 14.3 ± 3.3, 147.7 ± 11.5 and 551.7 ± 97.7 mg/m^3 for the control, low, intermediate and high concentration chambers, respectively (Table 2). The ratio of jet fuel aerosol to total jet fuel concentration was 0.07, 0.22 and 0.28 for the 200, 700 and 2000 mg/m^3 concentration chambers, respectively. Thus, as the total HEFA-F jet fuel concentration increased, the fraction of the total that existed as aerosol droplets increased.

An aerodynamic particle sizer was used to measure particle size distribution. Measurements were made by sampling from each chamber twice during the ten-day exposure period. The average mass median aerodynamic diameter and geometric standard deviation (MMAD (GSD)) of the aerosols were calculated as 1.4 (1.8), 1.4 (1.5), 1.6 (1.5) and 1.3 (1.4) µm for the control, low, intermediate and high concentration chambers, respectively (Table 2). Aerosols with particle size distributions between 1 and 4 µm are generally considered as respirable by rodents. More details may be found in Appendix A.

Table 2. Exposure Conditions for the Ten-Day Duration Groups

	Target Concentration	0 (mg/m^3)	200 (mg/m^3)	700 (mg/m^3)	2000 (mg/m^3)
Temperature (°F)	Mean of daily means	**70.7**	**70.2**	**68.3**	**71.6**
	SD	0.7	0.5	0.4	0.4
	Maximum daily mean	71.9	71.4	69.1	72.5
	Minimum daily mean	70.0	69.9	67.8	71.3
Relative Humidity (%)	Mean of daily means	**57**	**47**	**46**	**48**
	SD	5	3	4	3
	Maximum daily mean	66	53	55	55
	Minimum daily mean	52	45	42	45
Air Flow (L/min)	Mean of daily means	**274**	**225**	**225**	**225**
	SD	8	0	0	0
	Maximum daily mean	251	225	226	225
	Minimum daily mean	225	225	225	225
Actual Chamber Static Pressure (in H_2O)	Mean of daily means	**0.278**	**-0.369**	**-0.385**	**-0.165**
	SD	0.058	0.027	0.015	0.023
	Maximum daily mean	0.355	-0.331	-0.348	-0.102
	Minimum daily mean	0.178	-0.404	-0.402	-0.180
Actual Chamber Concentration (mg/m^3)	Mean of daily means	**0.0**	**201.5**	**685.3**	**1951.7**
	SD	0	8.1	23.2	71.8
	Maximum daily mean	0.0	209.0	729.6	2036.5
	Minimum daily mean	0.0	183.1	658.9	1844.8
Nominal Chamber Concentration (% target conc.)	Mean of daily means	**NA**	**114.9**	**98.7**	**107.1**
	SD	NA	3.6	4.5	6.6
	Maximum daily mean	NA	119.6	110.3	116.4
	Minimum daily mean	NA	107.7	94.8	93.4
Gravimetric Concentration (mg/m^3)	**Mean**	**0.116**	**14.255**	**147.716**	**551.729**
	SD	0.112	3.268	11.452	97.680
	Proportion total concentration	NA	0.071	0.215	0.283
Particle Size Distribution	MMAD (μm)	1.404	1.351	1.615	1.286
	GSD	1.756	1.456	1.491	1.432

Notes: conc. = concentration; GSD = geometric standard deviation; MMAD = mass median aerodynamic diameter; NA = not applicable; SD = standard deviation

4.3 Clinical Observations

There were no HEFA-F related clinical observations for either male or female rats in any concentration group during the course of the study. No unscheduled deaths occurred, nor were any animals found in a moribund condition. Individual animal clinical observation data are listed in Appendix B.

4.4 Body Weights

There were no statistically significant changes in male or female body weight means due to HEFA-F exposure following either the acute (six-hour) (Table 3), five-day, or ten-day durations (Table 4). Body weight means of rats after an eleven-day recovery following a single HEFA-F exposure also did not differ significantly. Statistically significant differences were observed in the mean body weights of the acute HEFA-F exposed male rats; however, differences were observed during the pre-exposure period and thus were not attributable to HEFA-F exposure. Individual bodyweight data are found in Appendix C.

4.5 Food and Water Consumption

Food and water consumption were measured in rats assigned to the ten-day exposure group during the two-day recovery period between the first and second weeks of HEFA-F exposure (five days per week). A decrease in mean food consumption was observed in the male rats of all HEFA-F exposure groups, but was only statistically significant ($p<0.05$) in the 2000 mg/m^3 group compared to control males (Table 5). An approximate 10 percent decrease in mean water consumption was observed in the female rats of all HEFA-F exposure groups (Table 5). Individual consumption data are located in Appendix D. Due to small group sizes and animal variability associated with food and water consumption measurements, these observed decreases may not be associated with HEFA-F exposure.

Table 3. Body Weight Data for Rats in the Acute (six-hour) and Acute plus Recovery (eleven-day) Exposure Groups

Males		Body Weight (g)									
		Acute (6-hour)				Acute + Recovery (11-day)					
Jet Fuel (mg/m^3)		(weighing day)				(weighing day)					
		-10	**-4**	**-1**	**2**	**-10**	**-4**	**-1**	**5**	**8**	**12**
0	Mean	116.58	153.90	162.52	165.72	111.16	146.56	156.22	172.98	181.38	202.34
	SD	7.019	5.800	6.701	5.534	6.137	8.677	8.130	8.958	8.551	9.820
	n	5	5	5	5	5	5	5	5	5	5
200	Mean	107.36	**143.84***	152.54	158.36	113.16	149.64	159.74	178.86	187.42	208.31
	SD	5.809	5.331	3.925	3.586	6.190	6.715	6.939	7.014	8.136	9.017
	n	5	5	5	5	5	5	5	5	5	5
700	Mean	109.84	**147.08***	156.86	159.52	113.78	150.08	158.06	173.22	183.10	198.62
	SD	6.581	7.007	8.616	8.490	5.867	7.671	8.291	8.871	9.718	12.057
	n	5	5	5	5	5	5	5	5	5	5
2000	Mean	108.34	**143.18***	**151.38***	**153.82***	107.74	142.98	151.24	165.42	172.94	190.70
	SD	6.606	7.775	7.807	7.480	4.531	4.417	6.007	6.906	7.197	7.459
	n	5	5	5	5	5	5	5	5	5	5
Females		**Body Weight (g)**									
		Acute (6-hour)				**Acute + Recovery (11-day)**					
Jet Fuel (mg/m^3)		**(weighing day)**				**(weighing day)**					
		-10	**-4**	**-1**	**2**	**-10**	**-4**	**-1**	**5**	**8**	**12**
0	Mean	113.06	129.76	130.34	129.56	112.22	127.68	127.88	136.18	140.04	149.92
	SD	2.524	3.450	3.955	4.275	2.883	3.884	3.567	3.858	3.595	4.271
	n	5	5	5	5	5	5	5	5	5	5
200	Mean	113.22	127.78	129.40	129.74	116.12	131.94	131.88	134.64	141.72	150.44
	SD	5.820	7.157	5.339	8.042	6.622	5.869	4.557	13.672	5.810	4.817
	n	5	5	5	5	5	5	5	5	5	5
700	Mean	114.98	128.00	128.38	128.40	115.16	130.66	131.42	139.84	142.90	153.48
	SD	6.800	4.304	3.434	3.323	3.088	5.442	4.970	4.476	5.567	7.386
	n	5	5	5	5	5	5	5	5	5	5
2000	Mean	120.18	134.74	134.56	133.96	113.20	129.00	130.64	136.20	141.42	151.98
	SD	5.212	6.445	7.057	6.576	8.048	8.220	7.482	6.172	8.194	6.334
	n	5	5	5	5	5	5	5	5	5	5

Note: *****Significance of $p < 0.05$ per Williams Test (Williams, 1971, 1977)

Table 4. Body Weight Data for Rats in the Five-Day and Ten-Day Duration Groups

Males		Body Weight (g)										
		Five-Day					Ten-Day					
Jet Fuel (mg/m^3)		(weighing day)					(weighing day)					
		-10	-4	-1	5	6	-10	-4	-1	5	8	13
0	Mean	108.76	147.02	155.78	171.80	167.18	108.30	144.26	152.50	168.38	176.02	176.83
	SD	6.166	9.329	10.645	9.684	10.796	7.241	10.376	11.625	12.490	13.277	16.341
	n	5	5	5	5	5	5	5	5	5	5	5
200	Mean	110.04	147.58	157.26	181.20	179.72	109.04	144.82	165.28	181.20	188.72	191.33
	SD	2.792	6.680	8.701	12.057	13.159	4.089	6.001	8.133	10.570	11.535	8.073
	n	5	5	5	5	5	5	5	5	5	5	5
700	Mean	107.86	146.46	156.46	176.02	175.30	114.08	151.70	161.22	181.18	191.52	191.17
	SD	3.048	4.072	4.300	6.621	5.797	3.932	6.228	6.853	8.634	9.023	10.609
	n	5	5	5	5	5	5	5	5	5	5	5
2000	Mean	111.92	146.96	157.52	168.28	167.39	111.94	147.40	157.84	171.12	182.24	180.48
	SD	9.824	9.545	9.321	7.642	10.535	3.798	4.372	4.571	2.283	2.746	6.398
	n	5	5	5	5	5	5	5	5	5	5	5
Females		**Body Weight (g)**										
		Five-Day					**Ten-Day**					
Jet Fuel (mg/m^3)		**(weighing day)**					**(weighing day)**					
		-10	**-4**	**-1**	**5**	**6**	**-10**	**-4**	**-1**	**5**	**8**	**13**
0	Mean	111.58	128.92	127.70	138.96	136.66	115.80	131.38	132.00	142.50	145.54	145.68
	SD	3.480	5.041	4.895	6.664	5.069	4.478	4.754	4.296	6.481	8.752	10.018
	n	5	5	5	5	5	5	5	5	5	5	5
200	Mean	116.34	131.16	132.54	142.64	137.08	118.90	132.52	131.86	141.74	143.82	145.82
	SD	5.612	4.692	2.652	3.125	1.985	5.953	5.639	5.513	4.970	5.737	4.683
	n	5	5	5	5	5	5	5	5	5	5	5
700	Mean	112.24	127.46	127.86	135.98	133.90	108.52	125.54	125.92	133.88	138.00	140.30
	SD	4.813	4.607	7.012	6.698	7.168	3.411	3.076	3.681	4.994	4.974	6.671
	n	5	5	5	5	5	5	5	5	5	5	5
2000	Mean	116.82	132.76	133.84	135.90	133.92	108.10	130.10	130.50	134.82	138.60	137.76
	SD	7.107	7.939	7.455	6.353	5.835	8.162	7.211	6.804	8.197	8.310	7.440
	n	5	5	5	5	5	5	5	5	5	5	5

Table 5. Group Mean Food and Water Consumption in Ten-Day Duration Groups

	Consumption Over Two-Day Break			
Exposure Group	**Exposure Concentration**			
Males			**Food[1]**	**Water[2]**
Ten-Day	0	Mean	155.1	23.7
		SD	48.2	1.1
		N	4	5
	200	Mean	119.3	26
		SD	49.2	2.8
		N	4	5
	700	Mean	106.8	25.5
		SD	71.1	2.7
		N	5	5
	2000	Mean	60.9*	25.1
		SD	13.3	2.5
		N	3	5
Females			**Food[2]**	**Water[1]**
Ten-Day	0	Mean	78.5	22.8
		SD	6.5	1.9
		N	5	5
	200	Mean	87.3	20.3*
		SD	21.8	0.7
		N	5	5
	700	Mean	87.8	19.3**
		SD	11.9	1.9
		N	5	5
	2000	Mean	75.2	19.5**
		SD	4.8	1.6
		N	5	5

Notes: Note: *Significance of $p < 0.05$ per Williams Test (Williams, 1971, 1977); ** Significance of $p < 0.01$ per Williams Test (Williams, 1971, 1977); [1]No transformation; [2]Rank transformation

4.6 Urinalysis

Semi-quantitative analyses for protein, glucose, ketones, urobilinogen, bilirubin, nitrite, pH, blood (hemoglobin) and leukocytes indicated few minor differences between the control and HEFA-F exposure groups in either male or female rats. For example, pH appeared slightly lower in HEFA-F exposed animals. Ketones and the presence of leukocytes and hemoglobin were slightly elevated in the 2000 mg/m^3 male rats. Urine specific gravity was similar between control and HEFA-F exposure groups for male or female rats (Table 6). Analysis of variance (ANOVA) p values were 0.18 and 0.21 for male and female rats, respectively. There were no statistically significant differences in mean water consumption between control and HEFA-F exposed rats (male, $p = 0.44$ and female, $p = 0.08$) during the approximate 17-hour urine collection period. Mean urine volume was also similar between control and HEFA-F exposed

rats (male or female) during the collection period. Individual animal data are shown in Appendix E.

Table 6. Urine Specific Gravity in the Ten-Day Duration Groups

Target Exposure Concentration (mg/m^3)	Mean (n = 4)	Standard Deviation
Males		
0	1.0265	0.0084
200	1.0333	0.0154
700	1.0220	0.0070
2000	1.0370	0.0048
Females		
0	1.0313	0.0065
200	1.0235	0.0065
700	1.0253	0.0098
2000	1.0330	0.0037

4.7 Clinical Pathology

The detailed report of clinical pathology results for animals assigned to the ten-day exposure period is presented in Appendix F. Serum potassium and phosphorus levels were elevated in the male rats of the 700 mg/m^3 group compared to control males. There were no HEFA-F exposure-related differences in blood chemistry endpoints for female rats. Since the changes observed in HEFA-F exposed male rats were not concentration-related, these effects were not considered to be associated with HEFA-F exposure.

4.8 Gross Pathology

In general, gross lesions were absent at necropsy. One female rat of the 2000 mg/m^3 five-day exposure group had a white focus on her lungs that was correlated microscopically as alveolus inflammation. Liver nodes (microscopically diagnosed as hepatodiaphragmatic nodules) were observed in four rats total.

Mean organ weight values are listed in Tables 7 and 8. Adrenal glands, right kidney, left kidney and liver were weighed from male and female rats. In male rats, a statistically significant increase (compared to control group mean) in absolute and relative (percent of body weight)

mean right kidney weights was observed in male rats following the ten-day of HEFA-F exposure. The increase was observed in all HEFA-F exposure groups with only a slight indication of a concentration relationship. Similar increases were observed in the mean left kidney weights of the ten-day HEFA-F exposed males, but the increases were not statistically significant except in the 2000 mg/m^3 group (Table 7). Mean left kidney weights of the five-day HEFA-F exposed males were slightly increased above control values, but without statistical significance. In general, this effect was not observed in the five-day and ten-day HEFA-F exposed female rats, but the mean relative left kidney weight of the 2000 mg/m^3 group was significantly higher than the control females following the ten-day exposure.

The statistically significant decreases in either mean relative kidney weights or mean relative liver weights in the acute (six-hour) HEFA-F exposed female rats compared to control females were not considered HEFA-F exposure-related, because there were no comparable changes in mean absolute organ weights or in mean body weights. Small group size may have contributed to this observation. The statistically significant decrease in mean absolute right kidney weight in the acute (six-hour) 2000 mg/m^3 exposed male rats was attributed to the decrease in mean body weight for this group. The same explanation applies to the decrease in mean absolute left kidney weight of the 2000 mg/m^3 acute plus recovery (eleven-day) group males. Individual animal organ weights are provided in Appendix G.

Table 7. Male Rat Organ Weight Data in HEFA-F Exposure Groups

Target Exposure (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Terminal Body Weight- Male				
0	165.72 ± 5.53	202.34 ± 9.82	167.18 ± 10.80	176.83 ± 16.34
200	158.36 ± 3.59	208.31 ± 9.02	179.72 ± 13.16	191.33 ± 8.07
700	159.52 ± 8.49	198.62 ± 12.06	175.3 ± 5.80	191.17 ± 10.61
2000	153.82 ± 7.48	190.7 ± 7.46	167.39 ± 10.54	180.48 ± 6.40
Adrenal Glands- Male				
0	0.0386 ± 0.0044	0.0414 ± 0.0074	0.0416 ± 0.0063	0.041 ± 0.133
200	0.0322 ± 0.0013	0.043 ± 0.0066	0.0448 ± 0.0059	0.0538 ± 0.0088
700	0.037 ± 0.0039	0.0432 ± 0.0075	0.0382 ± 0.0041	0.045 ± 0.0030
2000	0.0382 ± 0.0042	0.0388 ± 0.0064	0.0396 ± 0.0098	0.0442 ± 0.0054
Kidney, Right- Male				
0	0.648 ± 0.037	0.722 ± 0.054	0.632 ± 0.023	0.636 ± 0.052
200	0.6161 ± 0.017	0.75 ± 0.034	0.668 ± 0.061	0.74** ± 0.025
700	0.606* ± 0.050	0.734 ± 0.074	0.664 ± 0.026	0.7380** ± 0.051
2000	0.572** ± 0.028	0.672 ± 0.029	0.632 ± 0.053	0.732** ± 0.025
Kidney, Left- Male				
0	0.636 ± 0.039	0.75 ± 0.051	0.626 ± 0.027	0.664 ± 0.074
200	0.622 ± 0.022	0.782 ± 0.030	0.686 ± 0.064	0.722 ± 0.024
700	0.612 ± 0.055	0.734 ± 0.064	0.692 ± 0.030	0.742 ± 0.058
2000	0.596 ± 0.029	0.676* ± 0.028	0.678 ± 0.073	0.718 ± 0.032
Liver- Male				
0	7.176 ± 0.157	8.114 ± 0.431	7.16 ± 0.347	6.726 ± 0.894
200	7.21 ± 0.264	8.59 ± 0.390	8.106 ± 0.995	7.378 ± 0.819
700	7.188 ± 0.219	8.158 ± 0.678	7.894 ± 0.380	7.72 ± 1.053
2000	6.866 ± 0.478	7.572 ± 0.349	7.404 ± 0.569	7.484 ± 0.553

Notes: Data are expressed as mean organ weights ± SD in grams, where n=5 for all cohorts.
*Significance of $p<0.05$ per Williams Test (Williams, 1971, 1977); ** Significance of $p<0.01$ per Williams Test (Williams, 1971, 1977)

Table 8. Female Rat Organ Weight Data in HEFA-F Exposure Groups

Terminal Body Weight- Female				
0	129.56 ± 4.28	149.92 ± 4.27	136.66 ± 5.07	145.68 ± 10.02
200	129.74 ± 8.04	150.44 ± 4.82	137.08 ± 1.99	145.82 ± 4.68
700	128.4 ± 3.32	153.48 ± 7.39	133.90 ± 7.17	140.30 ± 6.67
2000	133.96 ± 6.58	151.98 ± 6.33	133.92 ± 5.84	137.76 ± 7.44
Adrenal Glands- Female				
0	0.0398 ± 0.0088	0.0470 ± 0.0065	0.0548 ± 0.0108	0.0518 ± 0.0055
200	0.0462 ± 0.0071	0.0490 ± 0.0007	0.0516 ± 0.0045	0.0464 ± 0.0074
700	0.0402 ± 0.0037	0.0546 ± 0.0039	0.0498 ± 0.0037	0.0470 ± 0.0076
2000	0.0468 ± 0.0056	0.0510 ± 0.0069	0.0502 ± 0.0058	0.0524 ± 0.0084
Kidney, Right- Female				
0	0.518 ± 0.011	0.560 ± 0.031	0.510 ± 0.035	0.548 ± 0.048
200	0.478 ± 0.046	0.558 ± 0.041	0.558 ± 0.033	0.516 ± 0.018
700	0.484 ± 0.018	0.572 ± 0.055	0.496 ± 0.035	0.516 ± 0.034
2000	0.518 ± 0.028	0.544 ± 0.035	0.524 ± 0.021	0.554 ± 0.039
Kidney, Left- Female				
0	0.520 ± 0.022	0.556 ± 0.021	0.530 ± 0.022	0.528 ± 0.053
200	0.474 ± 0.042	0.570 ± 0.035	0.552 ± 0.030	0.518 ± 0.034
700	0.478 ± 0.016	0.588 ± 0.041	0.502 ± 0.049	0.524 ± 0.039
2000	0.498 ± 0.050	0.544 ± 0.021	0.520 ± 0.021	0.562 ± 0.019
Liver- Female				
0	4.990 ± 0.236	5.662 ± 0.265	5.522 ± 0.322	5.180 ± 0.515
200	4.654 ± 0.427	5.512 ± 0.191	5.342 ± 0.556	4.892 ± 0.385
700	4.746 ± 0.165	5.832 ± 0.353	5.076 ± 0.368	5.048 ± 0.455
2000	4.984 ± 0.288	5.618 ± 0.266	5.192 ± 0.441	5.276 ± 0.354

Notes: Data are expressed as mean organ weights ± SD in grams, where n=5 for all cohorts.
*Significance of $p<0.05$ per Williams Test (Williams, 1971, 1977); ** Significance of $p<0.01$ per Williams Test (Williams, 1971, 1977)

4.9 Histopathology

In summary, microscopic changes associated with exposure to HEFA-F were observed in the nasal cavity, lungs and kidneys (male rats only). There were no HEFA-F exposure-related changes observed in the larynx, trachea, liver, spleen, heart or adrenals. Histopathological results for tissues in which no lesions were observed may be found in Appendix H.

4.9.1 Nasal Airways. Nasal squamous, transitional, or respiratory epithelial inflammation was generally seen in the nose tip or Level I (Figure 5) and was evident in control animals in addition to those exposed to HEFA-F. It occurred at a higher incidence and greater severity in animals

exposed to HEFA-F in comparison with controls, but did not appear to be concentration dependent. The highest severity level was only a 1.0, which is a minimal effect. Histopathological results for epithelial inflammation may be found in Appendix H.

Starting in Level II of the nose, changes induced by HEFA-F were characterized by olfactory epithelial degeneration (including necrosis). Degeneration was evident as a disruption and disorganization of the olfactory epithelium at the junction of the respiratory and olfactory epithelium in the dorsal meatus. The olfactory epithelium of the dorsal meatus itself was largely unaffected. At Level III, olfactory epithelial degeneration was evident in the nasal septal organ, higher on the septum and at the junction of olfactory and respiratory epithelium in the dorsal meatus. At Levels IV and V, there were multifocal scattered areas of degeneration of the olfactory epithelium on the turbinates and septum with the preservation of the integrity of the epithelium in the dorsal meatus. Olfactory epithelial inflammation was not observed in control rats.

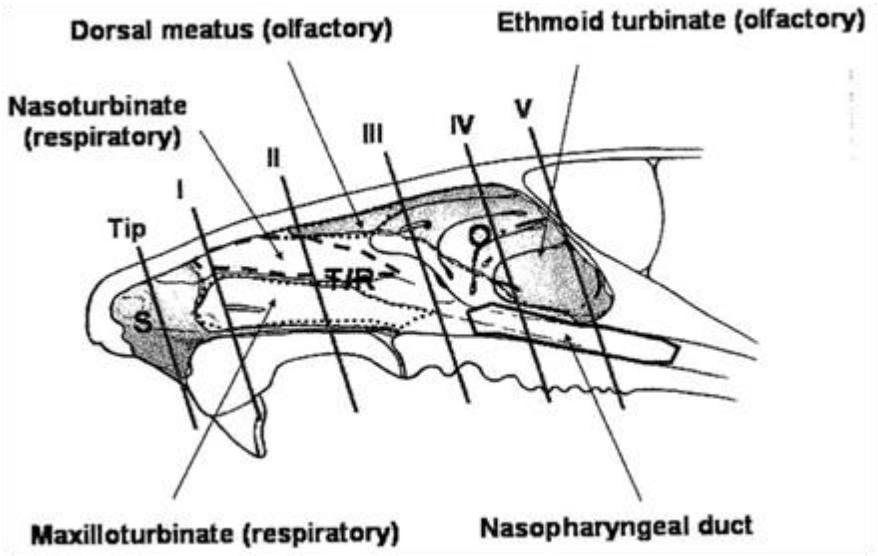


Figure 5. Nasal Passages of the Rat and Section Levels for Histopathology. Adapted from Morgan (1991; Figures 3 and 4)

In general, olfactory epithelial degeneration was observed in 40/40 rats exposed to 2000 mg/m^3 with an average degree of severity of 2 (slight/mild). Increase of exposure duration (acute to five-day to ten-day) or recovery (acute plus eleven-days) did not change the observed degree of severity in the high dose group rats (Tables 9 and 10). Similar results were observed in rats of the 700 and 200 mg/m^3 exposure groups, but the degree of severity of olfactory epithelial degeneration varied across duration compared to the 2000 mg/m^3 group. A dose-response increase in severity was seen only in the five-day exposed rats. There was a distinct decrease in incidence of this lesion in the eleven-day recovery animals of the low exposure group (200 mg/m^3) following a single six-hour exposure.

Table 9. Nasal olfactory epithelial degeneration in HEFA-F Exposure Groups by Nose Level in Male Rats

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)
200	Tip: 0 (0) I: 0 (0) **II: 3 (0.6)** **III: 5 (2)** **IV: 5 (1.8)** **V: 4 (1.4)**	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 3 (0.6)** IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (2)** **IV: 4 (0.8)** **V: 4 (0.8)**
700	Tip: 0 (0) I: 0 (0) **II: 3 (0.6**) **III: 5 (2)** **IV: 5 (2)** **V: 4 (1.6)**	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (2)** **IV: 4 (1)** **V: 4 (1)**	Tip: 0 (0) I: 0 (0) **II: 1 (0.2)** **III: 5 (1.2)** **IV: 5 (1)** **V: 5 (1.2)**	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (2)** **IV: 5 (2)** **V: 5 (1.4)**
2000	Tip: 0 (0) I: 0 (0) **II: 4 (0.8)** **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**	Tip: 0 (0) I: 0 (0) **II: 4 (1)** **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**	Tip: 0 (0) I: 0 (0) **II: 5 (1.2)** **III: 5 (2)** **IV: 5 (1.8)** **V: 5 (1.6)**	Tip: 0 (0) I: 0 (0) **II: 5 (1.6)** **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**

Notes: Degeneration includes necrosis. n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Table 10. Nasal olfactory epithelial degeneration in HEFA-F Exposure Groups by Nose Level in Female Rats

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Females				
0	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)
200	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**	Tip: 0 (0) I: 0 (0) II: 0 (0) III: 0 (0) IV: 0 (0) V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 2 (0.4)** **IV: 1 (0.2)** V: 0 (0)	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (1.6)** **IV: 5 (1)** **V: 5 (1)**
700	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (1.8)** **IV: 5 (1.2)** **V: 5 (1.2)**	Tip: 0 (0) I: 0 (0) **II: 1 (0.2)** **III: 5 (1.2)** **IV: 5 (1.2)** **V: 5 (1.2)**	Tip: 0 (0) I: 0 (0) II: 0 (0) **III: 5 (1.6)** **IV: 5 (1.2)** **V: 5 (1)**
2000	Tip: 0 (0) I: 0 (0) **II: 4 (0.8)** **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**	Tip: 0 (0) I: 0 (0) **II: 1 (0.2)** **III: 5 (2)** **IV: 5 (1.2)** **V: 5 (1.2)**	Tip: 0 (0) I: 0 (0) **II: 4 (0.8)** **III: 5 (2)** **IV: 5 (1.8)** **V: 5 (1.8)**	Tip: 0 (0) I: 0 (0) **II: 5 (1.2)** **III: 5 (2)** **IV: 5 (2)** **V: 5 (2)**

Notes: Degeneration includes necrosis. n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

4.9.2 Lung. Focal, chronic inflammation was observed in the lungs of four out of ten control rats at ten-days, no 200 or 2000 mg/m^3 HEFA-F exposed rats, and five out of ten 700 mg/m^3 HEFA-F exposed rats. In the other three time points, the pattern was even more inconsistent and tended to be higher in the controls and 200 mg/m^3 rats. There was no difference in males versus females for recovery, five-day and ten-day rats This type of inflammation involved discrete lesions which are seen as part of the background in control animals and are characterized by a focal lesion with alveolar macrophages with or without fibrosis of the interstitium. These lesions are found frequently, but not exclusively, in a subpleural location. Incidence and severity tables for this type of inflammation are found in Appendix H.

Two types of lung lesions were observed in HEFA-F exposed rats, but not air-exposed control rats. The incidence of interstitial fibrosis was nine out of ten (five-day) and ten out of ten (ten-day) in rats exposed to 2000 mg/m^3 HEFA-F, the only exposure group and duration of exposures to demonstrate this lesion (Table 11). Interstitial fibrosis was characterized by fibroblasts and collagen deposition in alveolar walls and within granulomas.

Table 11. Lung Interstitium Fibrosis in HEFA-F Exposure Groups

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	4 (0.8)	5 (1)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	5 (1)	5 (1)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Alveolus inflammation was characterized by an infiltration of macrophages, mononuclear cells and neutrophils. Granulomas consisting of aggregates of macrophages were observed, along with occasional observations of giant cells. The location of the inflammation was mainly in the pericentral alveoli around alveolar ducts. Among the ten-day exposure groups, there was no incidence in the 200 mg/m^3 HEFA-F rats while the incidence increase to 10/10 for the rats exposed to 700 and 2000 mg/m^3 HEFA-F (Table 12). However, the severity was only 1 (minimal) for the 700 mg/m^3 rats and increased to 2 (slight/mild) for the highest dose rats. The incidence and severity varied for the acute and five-day durations but tended to increase with increasing dose for both durations but did not reach the same severity value as the ten-day rats. For the acute plus recovery duration group, only one rat out of 30 HEFA-F exposed rats failed to reach full recovery (Table 12).

Due to the background inflammation observed in control rats, right cranial lobes of lungs from five HEFA-F exposed and five air-exposed (control) rats were stained with GMS and gram stains. No organisms were demonstrated (Appendix H).

Table 12. Alveolus Inflammation in HEFA-F Exposure Groups

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	1 (0.2)	0 (0)
700	2 (0.4)	1 (0.2)	4 (0.8)	5 (1)
2000	5 (1.8)	0 (0)	5 (2.2)	5 (2.6)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	2 (0.4)	0 (0)	0 (0)	0 (0)
700	4 (0.8)	0 (0)	3 (0.6)	5 (1)
2000	5 (2)	0 (0)	5 (2.2)	5 (2.6)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

4.9.3 Kidney. Hyaline droplets were observed in the kidneys of male rats exposed to 700 or 2000 mg/m^3 HEFA-F in either the five- or ten-day duration groups (Table 13). In general, the severity of hyaline droplets was graded as 1 (minimal).

Table 13. Hyaline Droplet Observation in Male Rat HEFA-F Exposure Groups

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	4 (0.8)	5 (1)
2000	0 (0)	0 (0)	5 (1)	5 (1)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

4.10 Lung Cytokine/Chemokine Screening

Following the ten-day exposure, only minimal changes in gene expression were observed at the lowest exposure concentration (200 mg/m^3). The predominant changes in gene expression occurred among the 2000 mg/m^3 exposure group and mainly involved chemokine genes. Male and female rats showed similar concentration response effects by exposure and duration groups, both in magnitude and direction of changes in gene expression. More details of this screening are found in Appendix I.

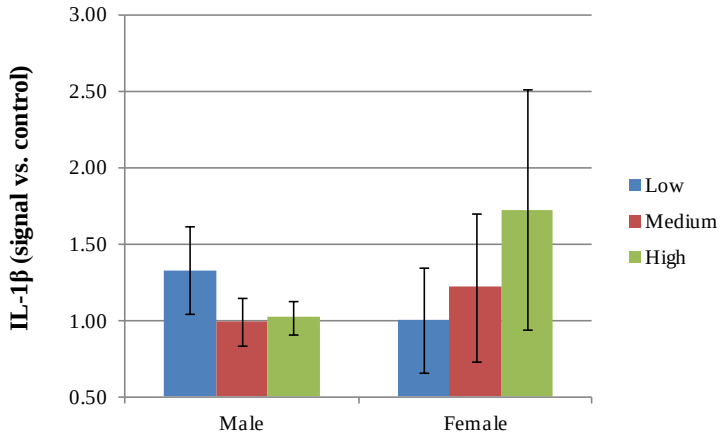
4.10.1 Concentration Response Summary: A single gene (Il1f6 = Interleukin 1 family member 6) was significantly expressed, as determined by Student's t-test ($p < 0.05$), in the lowest exposure group (200 mg/m^3). In the 700 mg/m^3 exposure group, there were 32 genes with significant differential expression and 53 in the 2000 mg/m^3 exposure group, with 29 of those being common to both concentrations. The magnitude of changes in gene expression at the 200 and 700 mg/m^3 concentrations was low; however, only a single gene (Spp1 = Secreted Phosphoprotein 1) showed a change greater than two-fold at 700 mg/m^3. There were 13 genes at 2000 mg/m^3 that had a greater change than two-fold, all with p-values much less than 0.05; all but one of these was a Chemokine Gene (CcHEFA-F is a chemokine receptor).

4.10.2 Gender Differences Summary: While there were differences in significance and expression level of some genes between sexes, the biological significance appears minimal. In both sexes, the predominant effect on gene expression was the effect of exposure concentration. Most changes in gene expression were again seen at the highest exposure concentration (2000 mg/m^3) and were predominantly among the Chemokine genes. These also tended to be the same genes with significance in the combined-gender concentration response analysis. Gender differences appear to be limited to relatively subtle differences in the expression intensity of significant genes.

4.11 Blood Cytokine Screening

In general, measurements of several cytokines associated with inflammatory response did not show a significant response to increasing exposure concentrations; however, there were some exceptions. At the 2000 mg/m^3 exposure concentration, female rats demonstrated a depression in IFN-γ expression. Female rats also presented a possible dose-response elevation in IL-1β and MCP-1 expression compared to control rats, as seen in Figure 6. The majority of results were lower than many of the standard curves generated as part of the testing, so the signal level produced by each result was compared to the control signal in order to determine variation to baseline. Results are provided as mean ratio to control, with control providing a value of 1.00. More details of this screening are found in Appendix I.

A



B

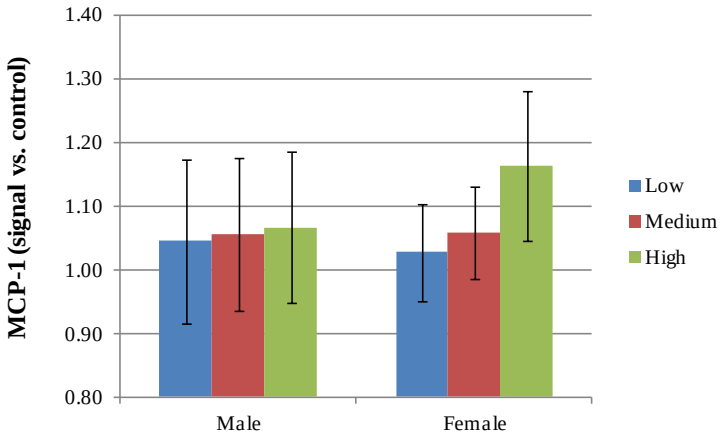


Figure 6. IL-1β (A) and MCP-1 (B) in the blood of male and female rats exposed to 200, 700 and 2000 mg/m^3 HEFA-F for a ten-day duration. Data are expressed as mean ratio to control, with control providing a value of 1.00; errors bars depict ± 1 SD.

4.12 Sensory Irritation Potential

A group of four male Swiss-Webster mice were exposed to HEFA-F at a target concentration of 2000 mg/m^{3}for 30 minutes. The actual measured concentration was 1916 mg/m^3 HEFA-F. The proportion of the total concentration that was aerosol was 21.2 percent. The mass median aerodynamic diameter ranged from 1.42 to 1.70 µm, which is a respirable size. The aerosol particle counts were relatively stable throughout the exposure, ranging from 576 to 737 particles/cc. Particle counts also indicate a rapid increase and decrease at exposure start and finish, respectively. Aerosol data are presented in Appendix J.

Individual breathing rate data are shown in Appendix J. The group mean respiratory rate decreased 23 percent from baseline values. The response of each animal was graded on a scale

of slight, moderate or extreme. A decrease in respiratory rate of 12 to 20 percent was graded as a slight response, 20 to 50 percent as a moderate response, and 50 to 85 percent as an extreme response. One mouse (# 901) was graded a slight response, showing little or no response while the other mice were graded a moderate response. The difference in response could not be explained by visual observations of the animals, so mouse # 901 was not excluded from the calculations. Mice that had greater response to the jet fuel showed decreased recovery (76, 87 and 96 percent of baseline) compared to the mouse that showed little response (125 percent of baseline).

The 30-minute exposure of mice to 1916 mg/m^3 HEFA-F evoked a slight to moderate upper airway sensory irritation with a group mean decrease in respiratory rate of 23 percent. Examination of the breathing pattern did not indicate a pulmonary irritation response.

5.0 DISCUSSION AND CONCLUSIONS

Fischer 344 rats were exposed by whole-body inhalation to combined aerosol and vapor concentrations of Hydroprocessed Esters and Fatty Acids from mixed animal fats and oils (HEFA-F) jet fuel for six hours per day for one- (acute), five- or ten-day durations. Target concentrations were 0 (control), 200, 700 or 2000 mg/m^3 HEFA-F. The average analytical total concentrations were 0 ± 0, 202 ± 8, 685 ± 23 and 1952 ± 72 mg/m^3 for the 0, 200, 700 and 2000 mg/m^3 exposure groups, respectively. The average aerosol concentrations were 0.12 ± 0.11 (background), 14.3 ± 3.3 (7 percent), 147.7 ± 11.5 (22 percent) and 551.7 ± 97.7 (28 percent) mg/m^3 for the 0, 200, 700 and 2000 mg/m^3 exposure groups, respectively. By comparison, aerosol concentrations were lower in a 90-day study of male and female rats exposed to 200, 700 and 2000 mg/m^3 SPK alternative jet fuel (0.6, 12 and 33 percent aerosol, respectively) (Mattie *et al.*, (2011a). A study of Jet A with two different groups of rats exposed to 2000 mg/m^3 had aerosol percents of 14 and 19 (Sweeney *et al.*, 2012). The Jet A tested was a blend of fuel from five petroleum companies and is JP-8 if military additives are included. HEFA-F is closer to the SPK aerosol fraction than Jet A; both alternative fuel aerosol proportions are much higher than Jet A.

There were no clinical observations or body weight changes attributed to HEFA-F exposure. Observed decreases in food and water consumption in HEFA-F exposed rats were not consistently significant compared to control groups and were considered random due to small group sizes. In urine samples collected from animals of the ten-day duration groups, pH appeared slightly lower in HEFA-F-exposed animals. Leukocytes were present with slightly elevated ketones and hemoglobin in the 2000 mg/m^3 male rats. Urine specific gravity was similar between control and HEFA-F exposure groups for both male and female rats. In blood samples collected at necropsy, serum potassium and phosphorus levels were elevated in the male rats of the 700 mg/m^3 group compared to control males. There were no HEFA-F exposure-related differences in blood chemistry endpoints in female rats. Since the changes observed in HEFA-F exposed male rats were not concentration-related, these effects were not considered to be associated with HEFA-F toxicity.

At necropsy, no gross lesions were attributed to HEFA-F exposure. In male rats, an increase (compared to control group mean) in absolute and relative mean right kidney weights was observed in male rats following the ten-day HEFA-F exposures. The increase was observed in all HEFA-F male exposure groups with a slight indication of being concentration-related. Mean left kidney weights of the five-day HEFA-F exposed males were slightly increased above control value, but without statistical significance. This effect was not observed in any of the five- and ten-day HEFA-F exposed female rats. Kidney weight increases are consistent with hyaline droplet formation, discussed below. There were no increases in kidney weights for male rats exposed to 0, 500, 1000 and 2000 mg/m^3 SPK jet fuel for ten days (Mattie *et al.*, 2011b). Sweeney *et al.* (2012) utilized only female rats in their ten-day study with Jet-A.

There were no HEFA-F exposure-related histopathological changes observed in the larynx, trachea, liver, spleen, heart or adrenals. Nasal cavities and lungs are not normally examined after an acute exposure or after the typical recovery period associated with the acute study. A five-day exposure is also not normally conducted or has histological examination of tissues. Nasal cavity changes induced by HEFA-F were characterized by olfactory epithelial degeneration observed in all rats exposed to 2000 mg/m^3 with an average degree of severity of slight/mild. For the acute exposure, olfactory degeneration occurred at all doses with little recovery at the highest dose, limited recovery at the middle dose and full recovery only at the lowest dose. Data suggest a nonreversible lesion or a need for a longer recovery period. More work may be needed in this area. Increase of exposure duration (acute to five- to ten-day) did not change the observed degree of severity. The same results were observed in rats of the 700 and 200 mg/m^3 exposure groups, but the degree of severity of olfactory epithelial degeneration was occasionally less compared to the 2000 mg/m^3 group. Minimal to mild olfactory epithelial degeneration was also observed in the nasal tissues of male and female rats exposed to 1000 mg/m^3 and 2000 mg/m^3 SPK concentrations for ten days (Mattie *et al.*, 2011b).

Squamous, transitional or respiratory epithelium inflammation was observed in the anterior region of the nasal cavity of both HEFA-F exposed and control animals. It occurred at a higher incidence and greater severity in animals exposed to HEFA-F in comparison to control animals. In general, the nasal cavity inflammation was more prevalent in male rats versus female rats. Focal chronic inflammation was also observed in the lungs of both HEFA-F exposed and control rats and was more common in males versus females in the acute study. Inflammation of the nasal cavities and chronic focal inflammation in the lungs was not seen in rats exposed to SPK fuel for ten days and appears to be an artifact of the current study (Mattie *et al.*, 2011b). The incidence of interstitial fibrosis was limited to rats exposed to 2000 mg/m^3 HEFA-F and only in the five- and ten-day duration of exposure. It is unclear what this means as interstitial fibrosis was not seen in rats exposed to SPK fuel for ten or 90 days (Mattie *et al.*, 2011a, 2011b).

The overall incidence of alveolus inflammation for all exposures including recovery was 30/40, 24/40, 3/40 and 0/40 in rats exposed to 2000, 700, 200 and 0 mg/m^3 HEFA-F, respectively, regardless of duration of exposure (acute, five- or ten-day). For the ten-day exposures, incidence was only in the 700 and 2000 mg/m^3 exposure groups, but was seen in all rats in these groups. The severity increased from minimal (1) at 700 to slight/mild (2.6) at 2000 mg/m^3 for both male and female rats. In the SPK ten-day exposure study, the incidence was 100 percent only in the 2000 mg/m^3 group, even though the middle dose was 1000 mg/m^3 (Mattie *et al.*, 2011b). In the

Jet A study reported by Sweeney *et al.* (2012), most of the lungs and nasal cavities were lavaged to look for markers of inflammation, so they were not available for histological examination. The lungs that were not lavaged did not show any pattern of alveolus inflammation. Monocyte chemoattractant protein 1 (MCP-1), a marker of inflammation, was elevated significantly in the 2000 mg/m^3 Sprague-Dawley rats but not the F344 rats after 14 days of exposure to Jet A. Alveolus inflammation in the lungs of rats after exposure to 2000 mg/m^3 jet fuel is a relatively consistent finding.

Hyaline droplets were observed in the kidneys of male rats exposed to 700 and 2000 mg/m^3 HEFA-F concentrations in either the five- or ten-day duration groups. The severity of hyaline droplets was graded as minimal. Hyaline droplets are consistent with the accumulation of a male rat-specific protein, alpha-2μ-globulin, in the kidney. JP-8 produced a strong dose dependent increase in hyaline droplets after 90-days (Mattie *et al.*, 1991) while in the kidneys of rats exposed to SPK for ten days, hyaline droplets were scored as mild to slight in all of the jet-fuel exposed male groups and did not show a correlation with jet fuel exposure concentration. Both HEFA-F and SPK jet fuels appear to be weak inducers of hyaline droplets, suggesting that they both lack the constituents in JP-8 that are strong inducers.

Results of the lung cytokine/chemokine analysis using PCR arrays indicated minimal changes in gene expression at the lowest exposure concentration of 200 mg/m^3 HEFA-F. The predominant changes in gene expression occurred at the highest exposure of 2000 mg/m^3 HEFA-F and mainly involved chemokine genes. Male and female rats showed similar exposure concentration-response effects, both in magnitude and direction of changes in gene expression. This was the first attempt to measure cytokine and chemokine gene expression in lung tissue exposed to a jet fuel. A more focused experiment may be required to observe changes in cytokines.

Analysis of proinflammatory cytokines in blood did not find any significant changes. This also may require a more focused animal study or *in vitro* studies to identify optimal cytokines and sampling time points to determine if cytokines are changing. It is also possible that HEFA-F did not produce sufficient toxicity to cause detectable changes in cytokines in the blood.

In addition to the rat inhalation exposures described above, sensory irritation potential was evaluated in mice as a single exposure limit test. The atmosphere was a combined vapor/aerosol that had a mean concentration of 1916 mg/m^3. HEFA-F evoked a slight to moderate sensory irritation with a group mean decrease in respiratory rate of 23 percent. A single animal (mouse 901) weighed 3 grams more than the specified weight range per the ASTM standard (2004) and was less responsive to the fuel than the other three mice. If this mouse is excluded from the data analysis, the average weight of the three remaining mice would be 27.7 g and the average group mean respiratory rate decreased 28.7 percent from baseline values.

The single, high exposure to HEFA-F (1916 mg/m^3) was targeted to correlate with the highest dose level in the rat exposures and to compare its relative irritancy with JP-8 (Whitman and Hinz, 2001), SPK (Hinz *et al.*, 2012) and two other HEFA jet fuels (to be tested in another study). Whitman and Hinz (2004) used a similar approach with four jet fuels to compare their relative irritation potential versus the naval jet fuel JP-5. The RD_{50} for SPK was 10,939 mg/m^3 (Hinz *et al.*, 2012) and for JP-8 it was 2876 mg/m^3 (Whitman and Hinz, 2001). The decrease in

respiratory rate of 23 or 28.7 percent at 1916 mg/m^3 suggests that the RD_{50} for HEFA-F may be between the values for JP-8 and SPK. Without additional values to show a slope, the RD_{50} cannot be determined.

This study helped to determine the ten-day inhalation toxicity of one HEFA jet fuel (HEFA-F) in order to compare results with the ten-day and 90-day SPK inhalation studies. A 90-day HEFA study was conducted for the HEFA jet fuel from camelina oil (HEFA-C) and will be described in a separate report. An objective of this study was to compare acute and short-term exposures to determine if they might be sufficient for assessing the inhalation hazard of alternative jet fuels. These studies showed that there are acute effects histopathologically that are not always representative of the effects seen after 90-days, the study duration typically used for setting occupational exposure limits. The five-day time is too short as it appears that effects are still developing. The ten-day exposure serves as a better range-finding study than a toxicity screening, because effects seen at this time point also are not always representative of the effects seen with other similar fuels after 90-days. Results of this study do indicate that more work could be performed to address acute lung effects versus long-term exposure.

6.0 REFERENCES

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APPENDIX A. EXPOSURE SUMMARY

Summary

F344 rats were exposed by whole body inhalation to a synthetic Jet Fuel called Hydroprocessed Esters and Fatty Acids-Mixed Fats (HEFA-F) generated as a mixture of aerosol and vapor. The concentration of HEFA-F Jet Fuel in the exposure chambers was monitored using infrared spectrophotometry and was recorded approximately every 30 minutes during each exposure period. The HEFA-F Jet Fuel exposure was conducted for 6 hours/day, 5 days/week for 2 weeks. The average daily mean (± standard deviations) was 0.0 (±0.0), 201.5 (± 8.1), 685.3 (±23.2), 1951.7 (±71.8) mg/m^3 for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

The environmental parameters specified in the protocol for temperature, relative humidity and airflows were maintained at or near the target set points of 72 ºF, 50 percent, and 225 L/min, respectively throughout the entire study. The exposures were performed form June 13, 2010 through June 24, 2010.

Introduction

Very limited toxicity testing of HEFA-F Jet Fuel has been performed. Since inhalation is a major route of exposure for other types of jet fuel, the assessment of toxicity of HEFA-F jet fuel by inhalation is needed to assess the risk of replacing or augmenting other types of jet fuel. This study was designed to assess the potential inhalation toxicity of the test substance when administered via inhalation exposure to Fisher 344 rats on a repeated basis for 5 days per week over two weeks. The assessment included clinical observations, urine collection and urinalysis, food and water consumption, gross pathology and histopathology.

This report describes the methods for HEFA-F Jet Fuel generation, analysis and exposure to facilitate the objectives.

Materials and Methods

Chemical

The test substance HEFA-F Jet Fuel (CAS No. 437986-20-4) was obtained from Syntroleum Corp. (Tulsa OK) by the sponsor. The terms alternative jet fuel, HEFA-F Renewable Jet Fuel, HEFA-F Jet Fuel and jet fuel are used synonymously in this report. The HEFA-F Jet Fuel was shipped in a five gallon drum by the sponsor to The Hamner Institutes and stored in a well ventilated area at room temperature.

Information regarding the synthesis of the jet fuel is maintained by the manufacturer. Information regarding composition and purity are maintained by the sponsor. The jet fuel was not diluted prior to use.

Chemical and Physical Properties of HEFA-F Jet Fuel [1]

Name:	HEFA-F Renewable Jet Fuel
Synonyms:	None
CAS No.:	437986-20-4
Molecular Formula:	NA [2]
Molecular Weight:	NA [2]
Specific Gravity:	Approximately 0.76
Flash Point:	100 – 125 ºF (PM)
Vapor Pressure:	20 deg C: ,0.3 kPa @ 20 ºC
Flammability:	NA [2]
Stability:	Stable under normal conditions of use and storage
Appearance:	Colorless Liquid
Storage Conditions:	Stored in cool, well-ventilated area away from all sources of ignition
Manufacturer:	Syntroleum Corporation, Tulsa, OK
Identity and Purity:	See manufacturer and Sponsor

[1] MSDS HEFA-F Renewable Jet Fuel, Revision Date 20-Aug-2007, Syntroleum Corporation, Tulsa OK
[2] NA: Not Available

Exposure Chamber

Animals (F344 rats) were housed in wire mesh cage units (R-24 cage units, Lab Products, Inc., Seaford DE). Each R-24 cage unit has 24 compartments that could hold the entire group of 20 males or 20 females for each exposure concentration. Up to 3 cage units with three feces catch pans can fit within a 1-m^3 stainless steel and glass inhalation exposure chamber with silicon door seals (H1000, Lab Products, Inc., Seaford DE). This study used four 1-m^3 exposure chambers, each with two cage units and three catch pans in place. Three chambers were used for the HEFA-F Jet Fuel target exposure concentrations and one chamber for the control. Three separate 1-m^3 chambers were used as housing chambers for holding the dosed groups after the exposures ended. The control group was exposed and housed in the same chamber. A supply fan, an exhaust fan and two butterfly dampers controlled airflow through the chamber. Airflow was monitored by measuring the pressure drop across an orifice on the inlet side of the chamber and controlled by the damper in the supply duct system.

Temperature and relative humidity were measured near the center top of the chamber by a Rotronic Humidity Sensor (Series 200, Rotronic Instrument Corp., Huntington NY) connected to the Continuum Building Automation System (Andover Controls Corporation, Andover MA).

Temperature was verified by comparing the ambient air temperature recorded by the probe to a certified mercury thermometer. The relative humidity sensor was verified by immersing the sensor probe in an atmosphere of known humidity generated from saturated salt solutions.

Generation System

The jet fuel was generated as a mixture of aerosol and vapor by pumping the liquid jet fuel into an air atomizing nozzle (Model SUJ1A with fluid cap 1650 and air cap 64, Spraying Systems Co., Wheaton IL). A liquid metering pump (Model QVG-50, FMI, Fluid Metering, Inc., Syosset NY) pumped liquid jet fuel from a glass bottle reservoir to the nozzle. Compressed instrument air at approximately 50 psi was supplied to the nozzle. The nozzle assembly was housed in a stainless steel sanitary tee fitting. The spray was directed into a custom-made glass mixing tube, consisting of a 165 mm outer diameter (OD, 6.5 in) by 610 mm (24 in) glass tube that tapered at both ends to a cylindrical glass tube 63.5 mm OD (2.5 in) by 25 mm (1 in) long for an overall length of approximately 864 mm (34 in). The glass mixing tube dimensions were designed to contain the jet plume for mixing with chamber air, while minimizing impaction or intersection of the jet plume with the walls of the tube. The total flow of the chamber passed through the glass mixing tube, carrying the generated jet fuel mixture into the exposure chamber. A separate generation system was used for each exposure concentration. The FMI pump flow rate was determined during pre-study testing. The FMI pump produced a pulsatile liquid flow and ultimately generated a pulsing aerosol concentration into the chamber air flow. The pulsations were of the order of seconds, so the mixing with the chamber air flow and subsequent residence time in the exposure chamber dampened the pulsations to the point where the aerosol concentration was stable.

Analytical Systems

The jet fuel test material is a mixture of organic compounds of varying volatility. In this study, it was generated as an aerosol using a spray nozzle. As droplets were formed, the more volatile components evaporated from the droplets resulting in a chamber atmosphere that was a mixture of liquid droplets and vapor. Both the vapor and aerosol in the exposure atmosphere were pulled through an unheated line into the heated sample cell of the infrared spectrophotometer where the aerosol evaporated. Thus, the spectrophotometer measured the total mass concentration. As the atmosphere was sampled for aerosol concentration, the equilibrium between the liquid aerosol and vapor could be altered. For example, droplets collected on a filter may continue to evaporate, reducing the measured aerosol concentration. On the other hand, vapor could condense onto the collected droplets, increasing the measured aerosol concentration. The sampling of an atmosphere of droplets and vapors for aerosol concentration has been an area of research (Volckens *et al.*, 1999). In that study, various methods, including gravimetric filter samples, for measuring aerosol concentration, were evaluated and the results indicated that at higher mist concentrations, the methods provided similar values. Concentrations used in this study were higher than in the aforementioned study, indicating that a gravimetric filter should provide adequate measurement of aerosol concentration. A gravimetric filter was used to sample the aerosol mass concentration.

An infrared spectrophotometer (MIRAN 1A, Foxboro Co., South Norwalk CT) was used to monitor the total concentration of jet fuel in the chamber. The sensing cell of the IR spectrophotometer was warmed to approximately 50 ºC by a heat tape connected to a variable transformer and covered by a sheet of neoprene rubber fabric for insulation. The temperature was monitored using a thermistor (ST-R3, Precon, Memphis TN) and was monitored by the Continuum Building Automation System. When the sample was pulled through heated cell, the aerosol droplets evaporated. A chart recorder was used to continuously record the electrical output of the IR spectrophotometer. The electrical output was also monitored by the Continuum Building Automation System for recording the concentration of the exposure report.

Operating Conditions for the Infrared Spectrophotometers

Target Concentration	0 (mg/m^3)	200 (mg/m^3)	700 (mg/m^3)	2000 (mg/m^3)
Serial No.	4121	4159	1865	4186
Wavelength (μm)	3.45	3.45	3.40	3.46
Pathlength (m)	2.25	12.75	2.25	2.25
Slit (mm)	1	1	1	1
Range	1A	1A	1A	1A
Response Time (sec)	1	1	1	X10
Course zero	X10	X10	X10	1
Calibration Curve	$y=1068.3x^2 + 4001.2x$	$y=160.24x^2 + 723.53x$	$y=1644.9x^2 + 3104.9$	$y=5547.3x^2 + 5166.6x$
Correlation Coefficient	0.9996	0.9998	1.0	0.9999
Calibration Range (mg/m^3)	0 to 336.9	0 to 336.9	0 to 943.3	0 to 3368.8
Estimated Limit of Detection	20 mg/m^3	4 mg/m^3	16 mg/m^3	26 mg/m^3

Particle size distribution measurement was conducted using an aerodynamic particle sizer (APS, Model 3321, TSI, Inc., Shoreview MN). The instrument was connected to a sample port on the chamber. Dilution air was added in order to keep the aerosol concentration out of overload conditions.

Mass weight (gravimetric) filter (MWF) samples were collected on glass fiber filters (47 mm diameter) a minimum of three times a week for the two week exposure period. A microbalance (Model C35, Cahn Instruments) was used to weigh the filters. A weighed filter was connected to a sample port on the chamber. After pulling a sample of the atmosphere through the filter at a

known flow rate and time, the filter was reweighed. Samples were obtained from the front of each target concentration chamber door during the exposure period.

Nominal concentration was calculated from the air flow rate through the chamber and the mass of material used each exposure day.

Calibration

The infrared spectrophotometer was calibrated using a closed loop method. A stainless steel diaphragm pump was connected to the inlet and outlet of the infrared spectrophotometer with specified lengths of tubing with a fitting containing a septum on the inlet side to produce a closed system with a specified volume. Clean air was circulated and a scan of response as a function of wavelength was conducted. A small sample of jet fuel was injected and a scan superimposed over the clean air scan to identify a representative peak. The infrared wavelength was set at the identified wavelength. Clean air was readmitted into the infrared spectrophotometer, and then jet fuel was injected in a series of volumes to produce a set of increasing concentrations of jet fuel. A calibration curve of spectrophotometer response as a function of jet fuel concentration was produced.

Each infrared spectrophotometer (IR) was calibrated by injecting a known volume of HEFA-F Jet Fuel into the heated closed loop of the IR analyzing cell, according to the following equation:

$$C_1 = \left(\frac{V_2 \times \rho}{V_1} \right) \times 10^3$$

Where:

C_1 = Concentration IR cell, (mg/m^3)
V_1 = Volume of IR Cell, (L)
V_2 = Volume of injection, (μL)
ρ = Density of test material (g/mL)

Estimated Limit of Detection

The limit of detection was estimated as the lowest voltage change detectable by the Continuum Building Automation System (0.005 volts). The limit of detection was then based on each MIRAN calibration curve.

Particle Size Distribution Measurements

An optical particle sizing spectrometer (Aerodynamic Particle Sizer, Model APS 3321, TSI, Inc., St. Paul MN) was used to measure particle distribution. The particle size distribution data using the optical particle sizer were collected from each of the chambers during the two week exposure period. The reported results were the average of data collection runs from the 2 sampling days.

Chamber Distribution

Prior to animals being placed in the chambers, each dosing chamber was checked for uniformity of distribution of the test compound by measuring the concentration at 9 locations within the chamber. The locations were at the corners of the rectangle defined by the animal cage unit for the two cage units used. Chamber distributions for these exposures were performed prior to the start of the study.

Concentration Uniformity in 1-m^3 Chambers

	Concentration (mg/m^3) Chamber		
Sample Position [2]	**405A [1]**	**405B [1]**	**405D [1]**
Center (Home [1])	197.3	674.7	1906.4
1	203.8	667.6	1973.2
2	208.7	692.6	1973.3
3	188.5	639.1	1832.1
Center	204.6	692.6	1906.4
4	184.5	649.8	1807.5
5	217.6	721.4	1931.4
6	219.2	746.7	1948.1
Center	208.7	721.4	1906.4
7	217.6	710.6	1964.8
8	211.1	692.6	2057.7
Center	211.1	721.4	1914.7
Within Port (WP) Average (Center Values)	205.4	702.5	1908.5
WP Std. Dev	6.0	23	4.15
Total Port (TP) Average (positions 1-8, TP Average)	206.3	691.4	1922
TP Std. Dev.	12.5	34.4	77.0
TPCV [3] (%)	6.06	4.98	4.00
WPCV [4] (%)	2.94	3.27	0.22
BPCV [5] (%)	5.30	3.75	4.00

[1] Chamber target exposure concentrations for chambers 405A, 405B, and 405D were 200, 700, and 2000 mg/m^3, respectively.
[2] Sample locations: 4 corners of rectangular cage units on middle and lower bottom levels and chamber center on middle level (Home).
[3] TPCV = Total Port Coefficient of Variation: (TP Std. Dev. / TP Average) * 100
[4] WPCV = Within Port Coefficient of Variation: (WP Std. Dev. / WP Average) * 100
[5] BPCV = Between Port Coefficient of Variation: $\sqrt{[(TPCV)^2 - (WPCV)^2]}$

Chamber Kinetics

As with previous studies, during pre-study testing, it was observed that the concentration of the test substance in the chamber did not follow the expected clearance kinetics when the generation system was shut off. The concentration of a compound typically decreases after generation ceases in a specified time that is determined by the chamber volume, flow rate through the chamber and mixing characteristics. For the jet fuel test material, the concentration decreased much more slowly, requiring hours to clear the exposure chamber.

To avoid exposing animals to the residual material in the chamber, the animals were transferred from the exposure chamber to similar 1-m^3 chambers for housing only. After exposures had ended, the 200 mg/m^3 chamber cleared for 30 minutes, and the 700 and 2000 mg/m^3 chambers cleared for 60 minutes. After the clearance period, cage units containing the animals were moved from exposure chambers to designated housing chambers.

Study Day

Study day numbering started from the first day of exposure and continued sequentially up to and including the day when the last animal was necropsied or otherwise removed from the study.

An exposure day for these exposures was defined as a 6-hour exposure from approximately 8:00 am until 2:00 pm. An exposure began when compressed air and jet fuel flow were applied to the spray nozzle, and ended when air and fuel to the nozzle were stopped (Cheng and Moss, 1995). Each exposure was followed by a 30-minute clearance period for the low concentration and 60-minute period for the mid and high concentration to allow the chemical to significantly reduce in the exposure chamber prior to opening for animal care procedures. The lighting cycle for the animals was 12 hours of light (7:00 am until 7:00 pm) followed by 12 hours of darkness and was controlled by the Continuum Building Automation System. The exposures were performed form June 13, 2010 through June 24, 2010.

Environmental Parameters

The Continuum Building Automation System recorded the average temperature, relative humidity, airflow and static pressure over a 30 minute period and a 24-hour report of the environmental parameters data was printed each day for each exposures day.

Domiciliary Housing

Animals were housed during non-exposure periods in 1-m^3 chambers of the same model as the exposure chambers. After the exposure and clearance period, cage units holding the animals were transferred to the housing chamber located in the same room. The control group was kept in the same chamber but serviced the same as the dose groups. Animals were provided with water *ad libitum* and food during non-exposure periods.

At the end of each exposure, a daily exposure report was printed that listed the sample time, temperature, relative humidity, chamber airflow, static pressure and concentration for the 6-hour exposure period. The daily mean, standard deviation, minimum and maximum values were determined. The reported study summary data (daily average mean, standard deviation, minimum and maximum values) for each of these parameters was determined from the average daily mean values.

Temperature, relative humidity, airflow and static pressure were printed each day for readings taken twice per hour for a 24-hour period. The mean, standard deviation, minimum and maximum values were determined. The reported study summary data (daily average mean, standard deviation, minimum and maximum values) for these parameters was determined from the average daily mean values.

Results

Chemical

The jet fuel was used as supplied by the Sponsor. No additional testing was completed.

Chamber Distribution

The coefficient of variation calculated for total port, between port and within port were less than 6.1 percent for all chambers, demonstrating that the test material was uniformly distributed throughout the chamber.

Exposure Conditions

The means and standard deviation were calculated from the average daily mean values for temperature, relative humidity, airflow, static pressure, and actual chamber concentration. The smallest minimum daily mean and the largest maximum daily mean are also shown. Results for these data are:

Temperature: The mean of the daily means (±) standard deviations for temperature were 70.7 (±0.7), 70.2 (±0.5), 68.3 (±0.4), 71.6 (±0.4) ºF for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Relative Humidity: The mean of the daily means (±) standard deviations for relative humidity were 57 (±5), 47 (±3), 46 (±4), 48 (±3) percent for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Air Flow: The mean of the daily means (±) standard deviations for chamber air flow were 247 (±8), 225 (±0), 225 (±0), 225 (±0) L/min for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Static Pressure: The mean of the daily means (±) standard deviations for static pressure were 0.278 (±0.058), -0.369 (±0.027), -0.385 (±0.015), -0.165 (±0.023) mm H_2O for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Concentration: The mean of the daily means (±) standard deviations for the measured total concentration were 0.0 (±0.0), 201.5 (± 8.1), 685.3 (±23.2), 1951.7 (±71.8) mg/m^3 for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively. The measured concentrations were at 114.9, 98.7, and 107.1 percent of the nominal concentrations for the target concentrations of 200, 700, and 2000 mg/m^3, respectively.

Except for the control group animals, which were held in the same chamber after the exposure period, all the other groups were moved into different holding chambers within the study exposure room. The data are the mean and standard deviation for average daily mean values for temperature, relative humidity, air flow and static pressure. The smallest minimum daily mean value and the largest maximum daily mean are also shown. The mean of the daily mean temperatures, relative humidity, air flows and static pressures for exposure chambers were maintained within the limits specified by the protocol.

Temperature: The mean of the daily means (± standard deviations) for temperature were 70.9 (±0.7), 67.9 (±0.5), 72.3 (±0.4), 72.1 (±0.6) ºF for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Relative Humidity: The mean of the daily means (±standard deviations) for relative humidity were 55 (±4), 53 (±2), 50 (±2), 55 (±4) percent for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Air Flow: The mean of the daily means (± standard deviations) for chamber air flow were 245 (±10), 225 (±0), 225 (±0), 224 (±2) L/min for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Static Pressure: The mean of the daily means (± standard deviations) for static pressure were 0.246 (±0.067), 0.281 (±0.100), 0.033 (±0.016), 0.100 (±0.161) mm H_2O for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively.

Summary of 1-m^{3}Chamber Operating Parameters for HEFA-F Jet Fuel Exposures

	Target Concentration	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
1-m^3 Temperature (ºF)	**Mean of daily means**	**70.7**	**70.2**	**68.3**	**71.6**
	Std Dev	0.7	0.5	0.4	0.4
	Maximum daily mean	71.9	71.4	69.1	72.5
	Minimum daily mean	70.0	69.9	67.8	71.3
	No. of Days	10	10	10	10
1-m^3 Relative Humidity (%)	**Mean of daily means**	**57**	**47**	**46**	**48**
	Std Dev	5	3	4	3
	Maximum daily mean	66	53	55	55
	Minimum daily mean	52	45	42	45
	No. of Days	10	10	10	10
1-m^3 Air Flow (L/min)	**Mean of daily means**	**274**	**225**	**225**	**225**
	Std Dev	8	0	0	0
	Maximum daily mean	251	225	226	225
	Minimum daily mean	225	225	225	225
	No. of Days	10	10	10	10
Actual Chamber Static Pressure (in H_20)	**Mean of daily means**	**0.278**	**-0.369**	**-0.385**	**-0.165**
	Std Dev	0.058	0.027	0.015	0.023
	Maximum daily mean	0.355	-0.331	-0.348	-0.102
	Minimum daily mean	0.178	-0.404	-0.402	-0.180
	No. of Days	10	10	10	10
Actual Chamber Concentration (mg/m^3)	**Mean of daily means**	**0.0**	**201.5**	**685.3**	**1951.7**
	Std Dev	0	8.1	23.2	71.8
	Maximum daily mean	0.0	209.0	729.6	2036.5
	Minimum daily mean	0.0	183.1	658.9	1844.8
	No. of Days	10	10	10	10
Nominal Chamber Concentration (mg/m^3)	**Mean of daily means**	**N/A**	**114.9**	**98.7**	**107.1**
	Std Dev	N/A	3.6	4.5	6.6
	Maximum daily mean	N/A	119.6	110.3	116.4
	Minimum daily mean	N/A	107.7	94.8	93.4
	No. of Days	N/A	10	10	10
Gravimetric Concentration (mg/m^3)	**Mean**	**0.116**	**14.255**	**147.716**	**551.729**
	Std Dev	0.112	3.268	11.452	97.680
	Proportion of total concentration	NA	0.071	0.215	0.283
Particle Size Distribution	MMAD (μm)	1.404	1.351	1.615	1.286
	GSD	1.756	1.456	1.491	1.432

Summary of 24-Hour Environmental Parameters for HEFA-F Jet Fuel Exposures

	Target Concentration	0 mg/m^3	200 mg/m^3	700 mg/m^3	2000 mg/m^3
24-Hour Temperature (ºF)	**Mean of daily means**	70.9	67.9	72.3	72.1
	Std Dev	0.7	0.5	0.4	0.6
	Maximum daily mean	72.1	69.2	73.2	73.4
	Minimum daily mean	70.2	67.4	71.9	71
	No. of Days	13	13	13	13
24-Hour Relative Humidity (%)	**Mean of daily means**	55	53	50	55
	Std Dev	4	2	2	4
	Maximum daily mean	62	56	53	59
	Minimum daily mean	50	50	47	42
	No. of Days	13	13	13	13
24-Hour Air Flow (L/min)	**Mean of daily means**	245	225	225	224
	Std Dev	10	0	0	2
	Maximum daily mean	250	225	225	225
	Minimum daily mean	225	225	224	219
	No. of Days	13	13	13	13
24-Hour Static Pressure (in H_20)	**Mean of daily means**	0.246	0.281	0.033	0.100
	Std Dev	0.067	0.100	0.016	0.161
	Maximum daily mean	0.345	0.398	0.045	0.481
	Minimum daily mean	0.094	0.082	0.003	-0.100
	No. of Days	13	13	13	13

Chamber Concentration

Particle size distribution was determined twice during the two week exposure period. A respirable aerosol was produced with a mass median aerodynamic diameter (MMAD) for the exposed groups at 1.351, 1.615, and 1.286 μm for the low, intermediate and high concentration groups.

The gravimetric filer samples were collected a minimum of 3 times a week for the two week exposure period. The results were 0.116, 14.255, 147.716, and 551.729 mg/m^3 for the target concentrations of 0, 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively. The ratio of jet fuel aerosol to total jet fuel concentration was 0.071, 0.215, and 0.283 for the target concentrations of 200, 700, and 2000 mg/m^3 HEFA-F Jet Fuel in air, respectively. Thus, as the total concentration of jet fuel increased, the portion of aerosol increased.

Aerosol Particle Size Distribution

Date	Location	Mass Aerodynamic Diameter (MMAD) (μM)	Geometric Standard Deviation (σ_g)
Control Chamber			
23-Jun-2010	Direct/Door	1.610	1.845
24-Jun-2010	Direct/Door	1.199	1.667
Daily Average Mean		1.404	1.756
Std Dev		0.294	0.126
Maximum daily mean		1.610	1.845
Minimum daily mean		1.199	1.667
No. of Days		2	2
200 mg/m^3 Chamber			
23-Jun-2010	Direct/Door	1.359	1.455
24-Jun-2010	Direct/Door	1.344	1.457
Daily Average Mean		1.351	1.456
Std Dev		0.010	0.001
Maximum daily mean		1.359	1.457
Minimum daily mean		1.344	1.455
No. of Days		2	2
700 mg/m^3 Chamber			
		Aerodynamic Diameter	Geometric Standard
23-Jun-2010	Direct/Door	1.662	1.498
24-Jun-2010	Direct/Door	1.569	1.483
Daily Average Mean		1.615	1.491
Std Dev		0.066	0.011
Maximum daily mean		1.662	1.498
Minimum daily mean		1.569	1.483
No. of Days		2	2
2000 mg/m^3 Chamber			
23-Jun-2010	Direct/Door	1.372	1.457
24-Jun-2010	Direct/Door	1.200	1.408
Daily Average Mean		1.286	1.432
Std Dev		0.122	0.035
Maximum daily mean		1.372	1.457
Minimum daily mean		1.200	1.408
No. of Days		2	2

Aerosol Mass Concentration

Date	Location	MWF (mg/m^3)
Control Chamber		
13-Jun-10	Direct/Door	0.361
15-Jun-10	Direct/Door	0.170
16-Jun-10	Direct/Door	0.125
20-Jun-10	Direct/Door	0.051
21-Jun-10	Direct/Door	0.013
22-Jun-10	Direct/Door	0.105
23-Jun-10	Direct/Door	0.080
24-Jun-10	Direct/Door	0.024
Daily Average Mean		0.116
Std Dev		0.112
Maximum daily mean		0.361
Minimum daily mean		0.013
No. of Days		8
200 mg/m^3 Chamber		
13-Jun-10	Direct/Door	10.218
15-Jun-10	Direct/Door	13.034
16-Jun-10	Direct/Door	11.430
20-Jun-10	Direct/Door	19.878
21-Jun-10	Direct/Door	15.583
22-Jun-10	Direct/Door	12.805
23-Jun-10	Direct/Door	13.288
24-Jun-10	Direct/Door	17.800
Daily Average Mean		14.255
Std Dev		3.268
Maximum daily mean		19.878
Minimum daily mean		10.218
No. of Days		8
700 mg/m^3 Chamber		
13-Jun-10	Direct/Door	153.615
15-Jun-10	Direct/Door	138.509
16-Jun-10	Direct/Door	132.672
20-Jun-10	Direct/Door	143.928
21-Jun-10	Direct/Door	141.510
22-Jun-10	Direct/Door	145.002
23-Jun-10	Direct/Door	159.400
24-Jun-10	Direct/Door	167.090
Daily Average Mean		147.716
Std Dev		11.452
Maximum daily mean		167.090
Minimum daily mean		132.672
No. of Days		8
2000 mg/m^3 Chamber		
13-Jun-10	Direct/Door	413.049
15-Jun-10	Direct/Door	398.315
16-Jun-10	Direct/Door	514.889
20-Jun-10	Direct/Door	607.745
21-Jun-10	Direct/Door	606.801
22-Jun-10	Direct/Door	604.052
23-Jun-10	Direct/Door	629.151
24-Jun-10	Direct/Door	639.832
Daily Average Mean		551.729
Std Dev		97.680
Maximum daily mean		639.832
Minimum daily mean		398.315
No. of Days		8

Inhalation Exposure Data

Date of Exposure	Study Day	Temperature (ºF)	Relative Humidity (%)	Air Flow (L/min)	Static Pressure (in of H_20)	Measured Chamber Concentration (mg/m^3)
Control (0.0 mg/m^3) Group						
13-Jun-10	1	71.9	66	225	0.178	0.00
14-Jun-10	2	71.1	52	250	0.353	0.00
15-Jun-10	3	71.4	55	249	0.282	0.00
16-Jun-10	4	70.9	63	250	0.316	0.00
17-Jun-10	5	70.9	52	251	0.355	0.00
20-Jun-10	8	70.3	57	248	0.220	0.00
21-Jun-10	9	70.3	56	250	0.228	0.00
22-Jun-10	10	70.1	56	250	0.256	0.00
23-Jun-10	11	70.0	57	250	0.299	0.00
24-Jun-10	12	70.0	56	250	0.295	0.00
Daily Average Mean		70.7	57	247	0.278	0.00
Std Dev		0.7	5	8	0.058	0.00
Maximum daily mean		71.9	66	251	0.355	0.00
Minimum daily mean		70.0	52	225	0.178	0.00
No. of Days		10	10	10	10	10
Low Concentration (200 mg/m^3) Exposure Group						
13-Jun-10	1	71.4	53	225	-0.331	196.2
14-Jun-10	2	70.4	45	225	-0.347	209.0
15-Jun-10	3	70.6	48	225	-0.398	208.7
16-Jun-10	4	70.1	52	225	-0.393	205.2
17-Jun-10	5	69.9	46	225	-0.366	207.7
20-Jun-10	8	70.0	46	225	-0.402	206.6
21-Jun-10	9	70.1	45	225	-0.404	201.1
22-Jun-10	10	69.9	46	225	-0.343	194.8
23-Jun-10	11	70.0	48	225	-0.354	183.1
24-Jun-10	12	70.0	46	225	-0.354	202.7
Daily Average Mean		70.2	47	225	-0.369	201.5
Std Dev		0.5	3	0	0.027	8.1
Maximum daily mean		71.4	53	225	-0.331	209.0
Minimum daily mean		69.9	45	225	-0.404	183.1
No. of Days		10	10	10	10	10

Inhalation Exposure Data (continued)

Date of Exposure	Study Day	Temperature (ºF)	Relative Humidity (%)	Air Flow (L/min)	Static Pressure (in of H_20)	Measured Chamber Concentration (mg/m^3)
Intermediate Concentration (700 mg/m^3) Exposure Group						
13-Jun-10	1	69.1	55	225	-0.385	713.0
14-Jun-10	2	68.3	42	225	-0.387	729.6
15-Jun-10	3	68.7	45	225	-0.395	690.1
16-Jun-10	4	68.3	51	225	-0.402	680.4
17-Jun-10	5	68.1	45	225	-0.348	664.2
20-Jun-10	8	67.8	46	226	-0.378	663.8
21-Jun-10	9	68.2	44	225	-0.382	677.0
22-Jun-10	10	68.0	44	225	-0.387	658.9
23-Jun-10	11	68.1	47	225	-0.389	674.3
24-Jun-10	12	68.2	44	225	-0.398	701.6
Daily Average Mean		68.3	46	225	-0.385	685.3
Std Dev		0.4	4	0	0.015	23.2
Maximum daily mean		69.1	55	226	-0.348	729.6
Minimum daily mean		67.8	42	225	-0.402	658.9
No. of Days		10	10	10	10	10
High Concentration (2000 mg/m^3) Exposure Group						
13-Jun-10	1	72.5	55	225	-0.160	1954.4
14-Jun-10	2	71.8	45	225	-0.165	1859.5
15-Jun-10	3	72.0	48	225	-0.166	1854.9
16-Jun-10	4	71.4	53	225	-0.173	2036.5
17-Jun-10	5	71.5	46	225	-0.175	1997.9
20-Jun-10	8	71.5	48	225	-0.171	1963.1
21-Jun-10	9	71.3	47	225	-0.102	1844.8
22-Jun-10	10	71.4	47	225	-0.178	1995.5
23-Jun-10	11	71.4	49	225	-0.178	1998.5
24-Jun-10	12	71.4	47	225	-0.180	2011.6
Daily Average Mean		71.6	48	225	-0.165	1951.7
Std Dev		0.4	3	0	0.023	71.8
Maximum daily mean		72.5	55	225	-0.102	2036.5
Minimum daily mean		71.3	45	225	-0.180	1844.8
No. of Days		10	10	10	10	10

24-Hour Environmental Data

Date of Exposure	Study Day	Temperature (ºF)	Relative Humidity (%)	Air Flow (L/min)	Static Pressure (in of H_20)
Control (0.0 mg/m^3) Exposure Group					
11-Jun-10	pre-study	70.5	51	225	0.094
12-Jun-10	pre-study	72.0	59	225	0.187
13-Jun-10	1	72.1	62	234	0.246
14-Jun-10	2	71.6	58	250	0.345
15-Jun-10	3	71.2	57	250	0.325
16-Jun-10	4	71.1	60	250	0.291
17-Jun-10	5	71.2	54	250	0.303
18-Jun-10	6	[1]	[1]	[1]	[1]
19-Jun-10	7	[1]	[1]	[1]	[1]
20-Jun-10	8	70.4	54	250	0.229
21-Jun-10	9	70.3	53	250	0.246
22-Jun-10	10	70.3	55	250	0.260
23-Jun-10	11	70.3	54	250	0.251
24-Jun-10	12	70.2	53	250	0.248
25-Jun-10	13	70.5	49.9	250	0.175
Daily Average Mean		70.9	55	245	0.246
Std Dev		0.7	4	10	0.067
Maximum daily mean		72.1	62	250	0.345
Minimum daily mean		70.2	50	225	0.094
No. of days		13	13	13	13
Low Concentration (200 mg/m^3) Exposure Group					
11-Jun-10	pre-study	67.9	50	225	0.101
12-Jun-10	pre-study	69.2	56	225	0.372
13-Jun-10	1	68.5	56	225	0.376
14-Jun-10	2	68.2	55	225	0.353
15-Jun-10	3	67.7	54	225	0.326
16-Jun-10	4	67.6	56	225	0.337
17-Jun-10	5	67.6	52	225	0.398
18-Jun-10	6	[1]	[1]	[1]	[1]
19-Jun-10	7	[1]	[1]	[1]	[1]
20-Jun-10	8	67.4	54	225	0.234
21-Jun-10	9	67.6	51	225	0.269
22-Jun-10	10	67.6	52	225	0.317
23-Jun-10	11	67.6	52	225	0.269
24-Jun-10	12	67.6	52	225	0.221
25-Jun-10	13	67.7	50	225	0.082
Daily Average Mean		67.9	53	225	0.281
Std Dev		0.5	2	0	0.100
Maximum daily mean		69.2	56	225	0.398
Minimum daily mean		67.4	50	225	0.082
No. of days		13	13	13	13

[1] Animals were removed from the chamber during this time for food and water consumption measurements

24-Hour Environmental Data (continued)

Date of Exposure	Study Day	Temperature (ºF)	Relative Humidity (%)	Air Flow (L/min)	Static Pressure (in of H_20)	Measured Chamber Concentration (mg/m^3)
Intermediate Concentration (700 mg/m^3) Exposure Group						
13-Jun-10	1	69.1	55	225	-0.385	713.0
14-Jun-10	2	68.3	42	225	-0.387	729.6
15-Jun-10	3	68.7	45	225	-0.395	690.1
16-Jun-10	4	68.3	51	225	-0.402	680.4
17-Jun-10	5	68.1	45	225	-0.348	664.2
20-Jun-10	8	67.8	46	226	-0.378	663.8
21-Jun-10	9	68.2	44	225	-0.382	677.0
22-Jun-10	10	68.0	44	225	-0.387	658.9
23-Jun-10	11	68.1	47	225	-0.389	674.3
24-Jun-10	12	68.2	44	225	-0.398	701.6
Daily Average Mean		68.3	46	225	-0.385	685.3
Std Dev		0.4	4	0	0.015	23.2
Maximum daily mean		69.1	55	226	-0.348	729.6
Minimum daily mean		67.8	42	225	-0.402	658.9
No. of Days		10	10	10	10	10

Date of Exposure	Study Day	Temperature (ºF)	Relative Humidity (%)	Air Flow (L/min)	Static Pressure (in of H_20)	Measured Chamber Concentration (mg/m^3)
High Concentration (2000 mg/m^3) Exposure Group						
13-Jun-10	1	72.5	55	225	-0.160	1954.4
14-Jun-10	2	71.8	45	225	-0.165	1859.5
15-Jun-10	3	72.0	48	225	-0.166	1854.9
16-Jun-10	4	71.4	53	225	-0.173	2036.5
17-Jun-10	5	71.5	46	225	-0.175	1997.9
20-Jun-10	8	71.5	48	225	-0.171	1963.1
21-Jun-10	9	71.3	47	225	-0.102	1844.8
22-Jun-10	10	71.4	47	225	-0.178	1995.5
23-Jun-10	11	71.4	49	225	-0.178	1998.5
24-Jun-10	12	71.4	47	225	-0.180	2011.6
Daily Average Mean		71.6	48	225	-0.165	1951.7
Std Dev		0.4	3	0	0.023	71.8
Maximum daily mean		72.5	55	225	-0.102	2036.5
Minimum daily mean		71.3	45	225	-0.180	1844.8
No. of Days		10	10	10	10	10

Deviations

The deviations noted for this inhalation exposure study were typically high humidity readings. These data represent deviations during at least one 30 minute (0.5 hour) monitoring period for a

24-hour period including exposure days. These deviations did not affect the integrity of the study.

Date	Parameter	Value Range (Low to High)	Type of Report [1]	Time Period (hours) [2]	Impact on Study (Yes/No)
0 mg/m^3 Exposure Group					
13-Jun-2010	Humidity	72.6 – 76.3	DER	3	No
13-Jun-2010	Humidity	72.6 – 75.7	EPR	2.5	No
16-Jun-2010	Humidity	70.2 – 71.9	EPR	1	No
700 mg/m^3 Exposure Group					
13-Jun-2010	Humidity	100	DER	0.2	No

[1]	EPR	24 Hour Environmental Parameter Report
	DER	Daily Exposure Report
[2]	EPR	Each reading represents 30 minute Average Value
	DER	Each reading represents Instantaneous Value

References

Cheng, Y.S., and Moss, O.R. (1995). Inhalation Exposure Systems, in Concepts in Inhalation Toxicology, 2nd Edition, R.O. McClellan and R.F. Henderson, eds., Taylor and Francis, Washington, DC.

Volckens, J., Boundy, M., Leith, D., and Hands, D. (1999). Oil Mist Concentration: A Comparison of Sampling Methods. Am. Ind. Hyg. Assoc. J. 60, 684-689.

APPENDIX B. INDIVIDUAL ANIMAL CLINICAL OBSERVATIONS

Males	**Clinical Observations**							
Time point	Dose Group	Animal Number	Clinical Sign	Day				
				-10	-4	-1	5	8
6 hr	0	101	No Abnormalities Detected	X	X	X	.	.
		102	No Abnormalities Detected	X	X	X	.	.
		103	No Abnormalities Detected	X	X	X	.	.
		104	No Abnormalities Detected	X	X	X	.	.
		105	No Abnormalities Detected	X	X	X	.	.
	200	106	No Abnormalities Detected	X	X	X	.	.
		107	No Abnormalities Detected	X	X	X	.	.
		108	No Abnormalities Detected	X	X	X	.	.
		109	No Abnormalities Detected	X	X	X	.	.
		110	No Abnormalities Detected	X	X	X	.	.
	700	111	No Abnormalities Detected	X	X	X	.	.
		112	No Abnormalities Detected	X	X	X	.	.
		113	No Abnormalities Detected	X	X	X	.	.
		114	No Abnormalities Detected	X	X	X	.	.
		115	No Abnormalities Detected	X	X	X	.	.
	2000	116	No Abnormalities Detected	X	X	X	.	.
		117	No Abnormalities Detected	X	X	X	.	.
		118	No Abnormalities Detected	X	X	X	.	.
		119	No Abnormalities Detected	X	X	X	.	.
		120	No Abnormalities Detected	X	X	X	.	.
6 hr + 11 days	0	301	No Abnormalities Detected	X	X	X	X	X
		302	No Abnormalities Detected	X	X	X	X	X
		303	No Abnormalities Detected	X	X	X	X	X
		304	No Abnormalities Detected	X	X	X	X	X
		305	No Abnormalities Detected	X	X	X	X	X
	200	306	No Abnormalities Detected	X	X	X	X	X
		307	No Abnormalities Detected	X	X	X	X	X
		308	No Abnormalities Detected	X	X	X	X	X
		309	No Abnormalities Detected	X	X	X	X	X
		310	No Abnormalities Detected	X	X	X	X	X
	700	311	No Abnormalities Detected	X	X	X	X	X
		312	No Abnormalities Detected	X	X	X	X	X
		313	No Abnormalities Detected	X	X	X	X	X
		314	No Abnormalities Detected	X	X	X	X	X
		315	No Abnormalities Detected	X	X	X	X	X
	2000	316	No Abnormalities Detected	X	X	X	X	X
		317	No Abnormalities Detected	X	X	X	X	X
		318	No Abnormalities Detected	X	X	X	X	X
		319	No Abnormalities Detected	X	X	X	X	X
		320	No Abnormalities Detected	X	X	X	X	X

Animal no longer alive

Males	**Clinical**							
Time point	Dose Group	Animal Number	Clinical Sign	Day				
				-10	-4	-1	5	8
5 days	0	501	No Abnormalities Detected	X	X	X	X	.
		502	No Abnormalities Detected	X	X	X	X	.
		503	No Abnormalities Detected	X	X	X	X	.
		504	No Abnormalities Detected	X	X	X	X	.
		505	No Abnormalities Detected	X	X	X	X	.
	200	506	No Abnormalities Detected	X	X	X	X	.
		507	No Abnormalities Detected	X	X	X	X	.
		508	No Abnormalities Detected	X	X	X	X	.
		509	No Abnormalities Detected	X	X	X	X	.
		510	No Abnormalities Detected	X	X	X	X	.
	700	511	No Abnormalities Detected	X	X	X	X	.
		512	No Abnormalities Detected	X	X	X	X	.
		513	No Abnormalities Detected	X	X	X	X	.
		514	No Abnormalities Detected	X	X	X	X	.
		515	No Abnormalities Detected	X	X	X	X	.
	2000	516	No Abnormalities Detected	X	X	X	X	.
		517	No Abnormalities Detected	X	X	X	X	.
		518	No Abnormalities Detected	X	X	X	X	.
		519	No Abnormalities Detected	X	X	X	X	.
		520	No Abnormalities Detected	X	X	X	X	.
10 days	0	701	No Abnormalities Detected	X	X	X	X	X
		702	No Abnormalities Detected	X	X	X	X	X
		703	No Abnormalities Detected	X	X	X	X	X
		704	No Abnormalities Detected	X	X	X	X	X
		705	No Abnormalities Detected	X	X	X	X	X
	200	706	No Abnormalities Detected	X	X	X	X	X
		707	No Abnormalities Detected	X	X	X	X	X
		708	No Abnormalities Detected	X	X	X	X	X
		709	No Abnormalities Detected	X	X	X	X	X
		710	No Abnormalities Detected	X	X	X	X	X
	700	711	No Abnormalities Detected	X	X	X	X	X
		712	No Abnormalities Detected	X	X	X	X	X
		713	No Abnormalities Detected	X	X	X	X	X
		714	No Abnormalities Detected	X	X	X	X	X
		715	No Abnormalities Detected	X	X	X	X	X
	2000	716	No Abnormalities Detected	X	X	X	X	X
		717	No Abnormalities Detected	X	X	X	X	X
		718	No Abnormalities Detected	X	X	X	X	X
		719	No Abnormalities Detected	X	X	X	X	X
		720	No Abnormalities Detected	X	X	X	X	X

Animal no longer alive

Females	**Clinical**							
Time point	Dose Group	Animal Number	Clinical Sign	Day				
				-10	-4	-1	5	8
6 hr	0	201	No Abnormalities Detected	X	X	X	.	.
		202	No Abnormalities Detected	X	X	X	.	.
		203	No Abnormalities Detected	X	X	X	.	.
		204	No Abnormalities Detected	X	X	X	.	.
		205	No Abnormalities Detected	X	X	X	.	.
	200	206	No Abnormalities Detected	X	X	X	.	.
		207	No Abnormalities Detected	X	X	X	.	.
		208	No Abnormalities Detected	X	X	X	.	.
		209	No Abnormalities Detected	X	X	X	.	.
		210	No Abnormalities Detected	X	X	X	.	.
	700	211	No Abnormalities Detected	X	X	X	.	.
		212	No Abnormalities Detected	X	X	X	.	.
		213	No Abnormalities Detected	X	X	X	.	.
		214	No Abnormalities Detected	X	X	X	.	.
		215	No Abnormalities Detected	X	X	X	.	.
	2000	216	No Abnormalities Detected	X	X	X	.	.
		217	No Abnormalities Detected	X	X	X	.	.
		218	No Abnormalities Detected	X	X	X	.	.
		219	No Abnormalities Detected	X	X	X	.	.
		220	No Abnormalities Detected	X	X	X	.	.
6 hr + 11 days	0	401	No Abnormalities Detected	X	X	X	X	X
		402	No Abnormalities Detected	X	X	X	X	X
		403	No Abnormalities Detected	X	X	X	X	X
		404	No Abnormalities Detected	X	X	X	X	.
			Pelage, Alopecia	.	.	.	.	X
		405	No Abnormalities Detected	X	X	X	X	X
	200	406	No Abnormalities Detected	X	X	X	X	X
		407	No Abnormalities Detected	X	X	X	X	X
		408	No Abnormalities Detected	X	X	X	X	X
		409	No Abnormalities Detected	X	X	X	X	X
		410	No Abnormalities Detected	X	X	X	X	X
	700	411	No Abnormalities Detected	X	X	X	X	X
		412	No Abnormalities Detected	X	X	X	X	X
		413	No Abnormalities Detected	X	X	X	X	X
		414	No Abnormalities Detected	X	X	X	X	X
		415	No Abnormalities Detected	X	X	X	X	X
	2000	416	No Abnormalities Detected	X	X	X	X	X
		417	No Abnormalities Detected	X	X	X	X	X
		418	No Abnormalities Detected	X	X	X	X	X
		419	No Abnormalities Detected	X	X	X	X	X
		420	No Abnormalities Detected	X	X	X	X	X

Animal no longer alive

Females	**Clinical**							
Time point	Dose Group	Animal Number	Clinical Sign	Day				
				-10	-4	-1	5	8
5 days	0	601	No Abnormalities Detected	X	X	X	X	.
		602	No Abnormalities Detected	X	X	X	X	.
		603	No Abnormalities Detected	X	X	X	X	.
		604	No Abnormalities Detected	X	X	X	X	.
		605	No Abnormalities Detected	X	X	X	X	.
	200	606	No Abnormalities Detected	X	X	X	X	.
		607	No Abnormalities Detected	X	X	X	X	.
		608	No Abnormalities Detected	X	X	X	X	.
		609	No Abnormalities Detected	X	X	X	X	.
		610	No Abnormalities Detected	X	X	X	X	.
	700	611	No Abnormalities Detected	X	X	X	X	.
		612	No Abnormalities Detected	X	X	X	X	.
		613	No Abnormalities Detected	X	X	X	X	.
		614	No Abnormalities Detected	X	X	X	X	.
		615	No Abnormalities Detected	X	X	X	X	.
	2000	616	No Abnormalities Detected	X	X	X	X	.
		617	No Abnormalities Detected	X	X	X	X	.
		618	No Abnormalities Detected	X	X	X	X	.
		619	No Abnormalities Detected	X	X	X	X	.
		620	No Abnormalities Detected	X	X	X	X	.
10 days	0	801	No Abnormalities Detected	X	X	X	X	X
		802	No Abnormalities Detected	X	X	X	X	X
		803	No Abnormalities Detected	X	X	X	X	X
		804	No Abnormalities Detected	X	X	X	X	X
		805	No Abnormalities Detected	X	X	X	X	X
	200	806	No Abnormalities Detected	X	X	X	X	X
		807	No Abnormalities Detected	X	X	X	X	X
		808	No Abnormalities Detected	X	X	X	X	X
		809	No Abnormalities Detected	X	X	X	X	X
		810	No Abnormalities Detected	X	X	X	X	X
	700	811	No Abnormalities Detected	X	X	X	X	X
		812	No Abnormalities Detected	X	X	X	X	X
		813	No Abnormalities Detected	X	X	X	X	X
		814	No Abnormalities Detected	X	X	X	X	X
		815	No Abnormalities Detected	X	X	X	X	X
	2000	816	No Abnormalities Detected	X	X	X	X	X
		817	No Abnormalities Detected	X	X	X	X	X
		818	No Abnormalities Detected	X	X	X	X	X
		819	No Abnormalities Detected	X	X	X	X	X
		820	No Abnormalities Detected	X	X	X	X	X

Animal no longer alive

APPENDIX C. INDIVIDUAL ANIMAL BODY WEIGHTS

Males	Individual Body Weights (g)										
	Dose	Animal	Day								
Timepoint	Group	Number	-10	-4	-1	2	5	6	8	12	13
6 hr	0	101	114.40	150.20	158.40	166.30	NM	NM	NM	NM	NM
		102	121.00	156.20	165.90	169.00	NM	NM	NM	NM	NM
		103	111.90	151.30	157.60	162.50	NM	NM	NM	NM	NM
		104	126.40	163.00	172.80	172.50	NM	NM	NM	NM	NM
		105	109.20	148.80	157.90	158.30	NM	NM	NM	NM	NM
	200	106	110.10	145.30	154.00	158.30	NM	NM	NM	NM	NM
		107	101.00	140.40	151.20	159.30	NM	NM	NM	NM	NM
		108	105.90	139.00	147.70	154.50	NM	NM	NM	NM	NM
		109	115.90	152.40	158.30	163.80	NM	NM	NM	NM	NM
		110	103.90	142.10	151.50	155.90	NM	NM	NM	NM	NM
	700	111	114.00	152.20	165.40	169.60	NM	NM	NM	NM	NM
		112	99.50	136.30	145.00	149.20	NM	NM	NM	NM	NM
		113	107.80	147.40	155.70	156.30	NM	NM	NM	NM	NM
		114	116.30	154.20	165.10	166.90	NM	NM	NM	NM	NM
		115	111.60	145.30	153.10	155.60	NM	NM	NM	NM	NM
	2000	116	98.80	132.90	142.10	145.90	NM	NM	NM	NM	NM
		117	117.30	154.70	163.70	165.50	NM	NM	NM	NM	NM
		118	108.70	143.20	151.70	155.00	NM	NM	NM	NM	NM
		119	107.20	141.40	150.00	153.70	NM	NM	NM	NM	NM
		120	109.70	143.70	149.40	149.00	NM	NM	NM	NM	NM
6 hr + 11 days	0	301	116.10	154.00	163.30	NM	184.00	NM	190.30	211.19	NM
		302	104.50	135.40	145.00	NM	163.20	NM	172.00	192.85	NM
		303	117.30	153.40	161.80	NM	178.30	NM	189.90	211.32	NM
		304	113.10	150.90	160.80	NM	174.90	NM	180.50	205.30	NM
		305	104.80	139.10	150.20	NM	164.50	NM	174.20	191.03	NM
	200	306	107.90	142.20	151.90	NM	167.00	NM	175.40	197.25	NM
		307	123.60	159.50	169.70	NM	183.30	NM	188.90	209.07	NM
		308	113.60	151.70	163.50	NM	182.60	NM	192.60	212.59	NM
		309	110.90	150.00	157.30	NM	177.90	NM	183.90	202.22	NM
		310	109.80	144.80	156.30	NM	183.50	NM	196.30	220.43	NM
	700	311	112.00	148.00	155.20	NM	171.40	NM	182.40	195.41	NM
		312	108.70	143.60	150.00	NM	163.80	NM	172.70	185.15	NM
		313	108.20	142.80	152.60	NM	168.10	NM	178.70	192.81	NM
		314	120.30	160.30	170.60	NM	187.00	NM	198.90	217.05	NM
		315	119.70	155.70	161.90	NM	175.80	NM	182.80	202.66	NM
	2000	316	108.50	140.80	148.90	NM	163.00	NM	172.50	190.06	NM
		317	106.60	148.00	158.80	NM	173.60	NM	178.70	193.78	NM
		318	101.20	137.50	143.00	NM	155.70	NM	161.80	179.50	NM
		319	113.80	147.00	154.90	NM	170.20	NM	179.90	200.02	NM
		320	108.60	141.60	150.60	NM	164.60	NM	171.80	190.12	NM

NM = not measured

Males	Individual Body Weights (g)										
	Dose	Animal	Day								
Timepoint	Group	Number	-10	-4	-1	2	5	6	8	12	13
5 days	0	501	110.20	148.60	155.80	NM	167.90	168.08	NM	NM	NM
		502	116.10	161.80	173.40	NM	189.00	184.57	NM	NM	NM
		503	108.80	141.80	150.80	NM	166.60	161.76	NM	NM	NM
		504	109.70	145.80	153.80	NM	169.20	165.76	NM	NM	NM
		505	99.00	137.10	145.10	NM	166.30	155.71	NM	NM	NM
	200	506	108.80	153.40	164.50	NM	190.80	191.44	NM	NM	NM
		507	108.40	141.50	150.70	NM	168.80	168.28	NM	NM	NM
		508	112.70	152.10	158.50	NM	184.00	180.91	NM	NM	NM
		509	113.30	151.70	166.40	NM	194.00	193.51	NM	NM	NM
		510	107.00	139.20	146.20	NM	168.40	164.46	NM	NM	NM
	700	511	106.60	143.40	152.80	NM	169.80	169.22	NM	NM	NM
		512	110.80	152.20	163.60	NM	183.30	182.66	NM	NM	NM
		513	110.80	149.30	157.20	NM	175.90	175.37	NM	NM	NM
		514	107.50	143.10	154.10	NM	169.10	170.03	NM	NM	NM
		515	103.60	144.30	154.60	NM	182.00	179.24	NM	NM	NM
	2000	516	121.70	157.50	166.00	NM	178.00	174.80	NM	NM	NM
		517	103.40	140.40	148.50	NM	160.60	158.63	NM	NM	NM
		518	122.30	154.50	166.20	NM	171.90	178.84	NM	NM	NM
		519	110.70	147.80	160.00	NM	170.50	170.40	NM	NM	NM
		520	101.50	134.60	146.90	NM	160.40	154.27	NM	NM	NM
10 days	0	701	120.70	162.10	172.70	NM	189.30	NM	198.50	NM	198.28
		702	105.40	138.60	146.20	NM	162.30	NM	166.70	NM	161.59
		703	101.90	135.90	143.90	NM	156.50	NM	166.50	NM	159.26
		704	107.70	143.60	151.40	NM	168.30	NM	177.00	NM	181.43
		705	105.80	141.10	148.30	NM	165.50	NM	171.40	NM	183.60
	200	706	111.70	149.30	161.70	NM	185.60	NM	193.30	NM	188.75
		707	109.70	145.90	156.90	NM	185.00	NM	190.00	NM	178.23
		708	114.00	150.50	164.90	NM	193.40	NM	203.40	NM	195.98
		709	105.40	135.50	143.80	NM	165.70	NM	172.00	NM	196.85
		710	104.40	142.90	154.10	NM	176.30	NM	184.90	NM	196.84
	700	711	115.00	152.80	160.30	NM	184.10	NM	194.90	NM	191.07
		712	107.80	140.90	149.70	NM	167.60	NM	176.90	NM	177.38
		713	118.20	156.90	166.60	NM	178.80	NM	192.40	NM	184.39
		714	113.70	153.70	165.20	NM	190.40	NM	201.50	NM	201.44
		715	116.20	154.20	164.30	NM	185.00	NM	191.90	NM	201.59
	2000	716	106.30	142.00	151.40	NM	172.60	NM	181.40	NM	176.84
		717	113.40	147.40	162.60	NM	173.50	NM	184.20	NM	186.20
		718	115.80	151.90	160.40	NM	171.90	NM	178.90	NM	173.14
		719	110.00	144.20	154.90	NM	167.90	NM	180.90	NM	178.10
		720	114.20	151.50	159.90	NM	169.70	NM	185.80	NM	188.11

NM = not measured

Females	Individual Body Weights (g)										
	Dose	Animal	Day								
Timepoint	Group	Number	-10	-4	-1	2	5	6	8	12	13
6 hr	0	201	115.70	135.20	131.90	135.30	NM	NM	NM	NM	NM
		202	110.80	125.90	124.20	125.30	NM	NM	NM	NM	NM
		203	113.90	129.90	133.90	131.00	NM	NM	NM	NM	NM
		204	110.20	128.00	128.70	125.30	NM	NM	NM	NM	NM
		205	115.20	129.80	133.00	130.90	NM	NM	NM	NM	NM
	200	206	116.60	133.60	133.50	136.40	NM	NM	NM	NM	NM
		207	121.90	136.90	136.10	139.60	NM	NM	NM	NM	NM
		208	109.10	125.70	126.60	126.80	NM	NM	NM	NM	NM
		209	108.90	120.70	127.90	125.80	NM	NM	NM	NM	NM
		210	109.60	122.00	122.90	120.10	NM	NM	NM	NM	NM
	700	211	110.30	130.40	130.40	130.30	NM	NM	NM	NM	NM
		212	117.00	130.50	130.80	131.10	NM	NM	NM	NM	NM
		213	118.90	132.30	131.30	130.90	NM	NM	NM	NM	NM
		214	122.80	122.60	123.80	123.90	NM	NM	NM	NM	NM
		215	105.90	124.20	125.60	125.80	NM	NM	NM	NM	NM
	2000	216	115.70	128.70	127.90	127.80	NM	NM	NM	NM	NM
		217	114.80	128.30	127.10	126.60	NM	NM	NM	NM	NM
		218	126.60	143.00	142.40	141.50	NM	NM	NM	NM	NM
		219	119.40	134.50	134.70	135.20	NM	NM	NM	NM	NM
		220	124.40	139.20	140.70	138.70	NM	NM	NM	NM	NM
6 hr + 11 days	0	401	112.20	125.10	127.80	NM	137.60	NM	141.30	150.90	NM
		402	108.00	123.20	122.80	NM	130.20	NM	134.50	144.10	NM
		403	114.00	127.70	127.10	NM	134.60	NM	138.60	147.10	NM
		404	115.60	133.30	132.70	NM	138.80	NM	142.20	153.10	NM
		405	111.30	129.10	129.00	NM	139.70	NM	143.60	154.40	NM
	200	406	110.60	133.90	133.60	NM	138.60	NM	138.50	149.10	NM
		407	114.70	128.20	129.60	NM	138.80	NM	138.40	146.00	NM
		408	119.00	135.40	133.70	NM	141.30	NM	146.60	156.30	NM
		409	126.10	138.40	137.20	NM	144.00	NM	149.20	154.70	NM
		410	110.20	123.80	125.30	NM	110.50	NM	135.90	146.10	NM
	700	411	115.90	135.50	133.70	NM	139.40	NM	146.20	158.90	NM
		412	114.40	131.30	132.10	NM	141.40	NM	144.90	154.00	NM
		413	110.20	121.90	122.70	NM	132.30	NM	133.00	141.10	NM
		414	117.60	134.80	134.80	NM	142.90	NM	144.70	154.00	NM
		415	117.70	129.80	133.80	NM	143.20	NM	145.70	159.40	NM
	2000	416	117.60	130.10	131.40	NM	137.00	NM	140.20	150.50	NM
		417	112.10	129.10	130.20	NM	133.10	NM	139.50	150.30	NM
		418	102.90	118.60	121.40	NM	130.10	NM	132.70	146.80	NM
		419	123.80	141.30	142.10	NM	146.30	NM	155.00	163.00	NM
		420	108.70	125.90	128.10	NM	134.50	NM	139.70	149.30	NM

NM = not measured

Females	Individual Body Weights (g)										
	Dose	Animal	Day								
Timepoint	Group	Number	-10	-4	-1	2	5	6	8	12	13
5 days	0	601	109.00	129.80	123.80	NM	132.20	134.10	NM	NM	NM
		602	107.90	121.00	122.10	NM	131.40	129.50	NM	NM	NM
		603	111.00	128.50	127.30	NM	141.70	137.80	NM	NM	NM
		604	116.50	134.90	132.90	NM	144.40	139.10	NM	NM	NM
		605	113.50	130.40	132.40	NM	145.10	142.80	NM	NM	NM
	200	606	109.30	125.50	128.90	NM	140.10	136.90	NM	NM	NM
		607	124.40	136.40	134.70	NM	144.50	137.90	NM	NM	NM
		608	115.40	131.80	134.80	NM	147.00	139.90	NM	NM	NM
		609	114.00	127.30	130.60	NM	139.50	134.60	NM	NM	NM
		610	118.60	134.80	133.70	NM	142.10	136.10	NM	NM	NM
	700	611	111.10	128.70	126.40	NM	136.40	135.50	NM	NM	NM
		612	107.50	122.30	123.30	NM	132.10	128.30	NM	NM	NM
		613	114.50	129.40	132.70	NM	137.30	135.90	NM	NM	NM
		614	108.70	123.40	119.80	NM	128.10	125.80	NM	NM	NM
		615	119.40	133.50	137.10	NM	146.00	144.00	NM	NM	NM
	2000	616	117.20	131.50	133.50	NM	131.80	129.90	NM	NM	NM
		617	113.60	126.20	126.20	NM	128.40	127.70	NM	NM	NM
		618	117.10	139.60	140.50	NM	144.20	141.90	NM	NM	NM
		619	127.80	142.20	142.20	NM	140.20	137.80	NM	NM	NM
		620	108.40	124.30	126.80	NM	134.90	132.30	NM	NM	NM
10 days	0	801	120.80	136.50	134.90	NM	150.00	NM	157.90	NM	162.90
		802	120.20	135.20	136.50	NM	145.60	NM	145.20	NM	144.70
		803	113.70	131.10	132.40	NM	143.80	NM	147.00	NM	142.00
		804	113.70	129.50	130.80	NM	140.40	NM	144.30	NM	141.80
		805	110.60	124.60	125.40	NM	132.70	NM	133.30	NM	137.00
	200	806	128.10	140.70	140.30	NM	148.60	NM	151.40	NM	152.30
		807	112.20	125.80	126.80	NM	135.20	NM	135.70	NM	140.50
		808	120.30	134.10	133.30	NM	143.60	NM	145.50	NM	146.70
		809	115.80	129.00	127.10	NM	139.40	NM	141.70	NM	142.10
		810	118.10	133.00	131.80	NM	141.90	NM	144.80	NM	147.50
	700	811	107.00	126.60	124.70	NM	132.90	NM	137.50	NM	144.50
		812	104.00	121.10	120.50	NM	128.40	NM	130.20	NM	134.60
		813	110.80	128.10	127.30	NM	133.90	NM	140.30	NM	136.50
		814	108.00	123.70	126.60	NM	132.20	NM	138.30	NM	135.90
		815	112.80	128.20	130.50	NM	142.00	NM	143.70	NM	150.00
	2000	816	99.40	134.80	135.30	NM	142.50	NM	147.50	NM	144.50
		817	104.20	118.40	118.90	NM	121.00	NM	125.10	NM	125.40
		818	114.90	130.00	130.70	NM	137.40	NM	139.00	NM	139.30
		819	103.50	130.20	132.10	NM	138.30	NM	142.40	NM	142.30
		820	118.50	137.10	135.50	NM	134.90	NM	139.00	NM	137.30

NM = not measured

APPENDIX D. INDIVIDUAL ANIMAL FOOD AND WATER CONSUMPTION FROM DAY 5 TO 8

Time point	Dose Group	Animal Number	Food	Water
Males				
10 days	0	701	25	23.33
		702	34.33	24.2
		703	16.53	24.6
		704	31.13	24.27
		705	0*	21.9
	200	706	1.33*	29.27
		707	24.2	27.37
		708	31.5	27.2
		709	8.07	22.13
		710	25.6	24.2
	700	711	30.47	30.23
		712	6.37	25.5
		713	16.1	24.1
		714	9.67	23.37
		715	37.73	24.53
	2000	716	8.73	24.53
		717	0*	27.13
		718	1.27*	21.63
		719	13.17	27.9
		720	10.3	24.07
Females				
10 days	0	801	12.97	25.17
		802	11.77	23.5
		803	12.17	23.53
		804	10.67	21.33
		805	9.17	20.33
	200	806	12.2	20.33
		807	17.07	19.5
		808	10.8	19.83
		809	11.17	21.3
		810	10.77	20.4
	700	811	14.57	22.17
		812	9.8	17.5
		813	11.4	18.6
		814	11.6	18.03
		815	12.37	20.37
	2000	816	11.5	21.43
		817	8.9	17.87
		818	9.47	20.87
		819	11.2	19.1
		820	10.43	18.33

*Data excluded from means due to spillage/malfunction

APPENDIX E. INDIVIDUAL ANIMAL URINALYSIS

Males			
Dose (mg/m^3)	Animal #	Urine vol (mL)	Water vol (mg)
0	701	16	16.7
	702	17	21.9
	703	11	17.6
	704	10	17.1
	mean	**13.5**	**18.3**
	SD	**3.5**	**2.4**
200	706	14	12.7
	707	5	7.8
	708	20	22.1
	709	17	21.9
	mean	**14**	**16.1**
	SD	**6.5**	**7.1**
700	711	17	24.2
	712	15	20.2
	713	23	29.5
	714	17	26
	mean	**18**	**25.0**
	SD	**3.5**	**3.9**
2000	716	9	18.1
	717	10	22.6
	718	11	18
	719	10	22.6
	mean	**10**	**20.3**
	SD	**0.8**	**2.6**

Females			
Dose (mg/m^3)	Animal #	Urine vol (mL)	Water vol (mg)
0	801	9	20
	802	8	15.6
	803	15	19.4
	804	8	12.7
	mean	**10**	**16.9**
	SD	**3.4**	**3.4**
200	806	19	26.4
	807	14	22
	808	8	14.3
	809	13	18.7
	mean	**13.5**	**20.4**
	SD	**4.5**	**5.1**
700	811	22	33.3
	812	10	17
	813	20	23.3
	814	9	17.2
	mean	**15.25**	**22.7**
	SD	**6.7**	**7.6**
2000	816	9	19.9
	817	8	16.5
	818	12	20.1
	819	10	18.5
	mean	**9.75**	**18.8**
	SD	**1.7**	**1.7**

Chemstrip 9

Possible results

Leucocytes neg./ trace/ +/ ++
Nitrite neg./ pos.
pH 5/ 6/ 7/ 8/ 9
Protein neg./ trace/ +30/ ++100/ +++500mg/dl
Glucose normal/ 50/ 100/ 250/ 500/ 1000mg/dl

Ketones neg./ +smal/ ++mod./ +++large
Urobiinogen normal/ 1/ 4/ 8/ 12mg/dl
Bilirubin neg./ +/ ++/ +++
Blood neg./ trace/ about 50/ about 250 Ery/ml

Male Rats

Animal # →	701	702	703	704	706	707	708	709	711	712	713	714	716	717	718	719
Chemstrip 9																
Leucocytes	Neg	Neg	Neg	Neg	Neg	Neg	+	Neg	Neg	Neg	Neg	Neg	trace	trace	Neg	trace
Nitrite	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
pH	7	7	7	6	6	6	5	5	7	7	7	6	6	6	6	6
Protein	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	trace	Neg	trace
Glucose	Normal	Normal	50	Normal	Normal	50	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Ketones	Neg	Neg	Neg	+	Neg	+	Neg	Neg	Neg	Neg	Neg	Neg	+	+	+	+
Urobiinogen	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Bilirubin	Neg	Neg	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*	Normal*
Blood	Neg	Trace	Trace	Neg	trace	trace	trace	trace	trace	trace	trace	about 250 Ery/ul	about 50 Ery/ul	about 50 Ery/ul	trace	about 50 Ery/ul
Spcific gravity	1.027	1.015	1.029	1.035	1.023	1.056	1.029	1.025	1.023	1.025	1.012	1.028	1.041	1.030	1.039	1.038

* Neg CMB 7/27/10 (RE)

Female Rats

Animal # →	801	802	803	804	806	807	808	809	811	812	813	814	816	817	818	819
Chemstrip 9																
Leucocytes	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Nitrite	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg
pH	6	7	7	6	6	7	7	5	6	5	5	6	6	6	5	6
Protein	Normal***	Trace	Trace	Trace	Normal**	trace	trace	Normal**	Normal**	Normal**	trace	trace	trace	trace	trace	Trace
Glucose	Normal	Normal	Normal	50	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Ketones	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Urobiinogen	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Bilirubin	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Blood	Neg	Neg	Trace	about 50 Ery/ul	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Spcific gravity	1.034	1.037	1.022	1.032	1.015	1.022	1.028	1.029	1.016	1.031	1.018	1.036	1.037	1.033	1.034	1.028

Normal = Negative for protein CMB 7/27/10 (CF)

APPENDIX F. CLINICAL PATHOLOGY

Report of Clinical Pathology Results
for
Acute and Ten-Day Inhalation Study of Alternative Jet Fuel
The Hamner Institutes for Health Sciences Protocol 10006
Antech Diagnostics
507 Airport Blvd., Suite 113
Morrisville, NC 27560
For
The Hamner Institutes for Health Sciences
6 Davis Drive
Research Triangle Park, NC 27709

Written and approved by:

Doug Neptun, Laboratory Director

8/27/10
Date

Antech Diagnostics GLP
507 Airport Blvd, Suite 113
Morrisville, NC 27560

I. Introduction

Male and female rats, Fischer (CDF™)[F344/DuCrl], were exposed to 0 (Room air), 200, 700 or 2000 mg/m^3 of Alternative Jet Fuel in an aerosol/vapor combination. Animals from time point 4 were evaluated for clinical pathology toxicologic effects at the terminal sacrifice.

II. Study Design and Methods

Male and female rats were divided into four exposure groups for clinical pathology analyses. Blood was collected from all rats at terminal sacrifice. Blood samples were transported to Antech Diagnostics as refrigerated samples. All samples were evaluated for clinical chemistry analysis. Results were entered into the ClinAxys v2.2 computer system. Clinical chemistry testing was performed using Olympus reagents and the Olympus 640e clinical chemistry analyzer (Center Valley PA). Tests performed by the Olympus included: albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (BUN), calcium (CA), cholesterol (CHOL), chloride (CL), creatine kinase (CPK), gamma-glutamyltransferase (GGT), glucose (GLU), lactate dehydrogenase (LDH), potassium (K), sodium (NA), phosphorus (PHOS) total bilirubin (TBIL), total protein (TPRO) and triglycerides (TRIG).

Statistical evaluation of the data was performed using SigmaStat software. The data was analyzed for normality followed by an ANOVA ($p<0.05$) and, if significant, a comparison of groups by Holm-Sidak. If the test for normality failed, the ANOVA was based on Kruskal-Wallis ANOVA on Ranks ($p<0.05$) and, if significant, Dunn's comparison of groups. Values are reported as mean, standard deviation (SD) and number of samples (n).

Ill. Results

Discussion of results refers to a comparison of the group mean to the control group mean. Unless otherwise stated, the difference is a statistically significant difference. Potassium and phosphorus were increased in the 700 mg/m^3 groups of male rats. There were no treatment-related differences for female rats. The changes noted in the male rats were not dose-related since the effect was not noted in the 2000 mg/m^3 group.

IV. Conclusions

There were no biologically significant or toxicologically significant changes in clinical chemistry values from exposure to Alternative Jet Fuel in an aerosol/vapor combination.

Abbreviations and Test Codes found in Clinical Chemistry Summary Tables

1F to 4F	groups of females
1M to 4M	groups of males
ALB	albumin
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	urea nitrogen
CA	calcium
CHOL	cholesterol
CL	chloride
COM	comment
CPK	creatine kinase
dL	deciliter
fL	fentoliters
HEM	hemolyzed
g	grams
GGT	gamma-glutamyl transferase
GLU	glucose
K	potassium
LDH	lactate dehydrogenase
mg	milligrams
mL	milliliter
mmol/L	millimoles/liter
n	number of samples in group
NA	sodium
pg	picograms
PHOS	inorganic phosphorus
QNS	quantity not sufficient for analysis
SD	standard deviation
sec	seconds
TBIL	total bilirubin
TPRO	total protein
TRIG	triglyceride
U/L	units/liter
uL	microliter

Study: 10006 Species: RAT
Time point: TIME 4

Group		BUN mg/dl	GLU mg/dl	NA mmol/L	K mmol/L
1M CONTROL	Mean	19	201	143	5.4
	SD	3.3	23.2	1.5	0.48
	n	5	5	5	5
2M 200 MG/M3	Mean	22	241	144	6.1
	SD	2.7	32.4	1.9	0.25
	n	5	5	5	5
3M 700 MG/M3	Mean	19	233	143	6.4 *
	SD	2.0	36.1	0.5	0.81
	n	5	5	5	5
4M 2000MG/M3	Mean	20	179	144	5.0
	SD	1.9	21.9	1.4	0.55
	n	5	5	5	5

Group		CL mmol/L	ALP U/L	ALT U/L	AST U/L
1M CONTROL	Mean	100	270	46	112
	SD	0.7	71.4	8.6	39.6
	n	5	5	5	5
2M 200 MG/M3	Mean	99	261	47	104
	SD	2.0	51.9	6.5	13.0
	n	5	5	5	5
3M 700 MG/M3	Mean	99	250	51	105
	SD	1.1	39.4	10.6	35.6
	n	5	5	5	5
4M 2000MG/M3	Mean	100	274	42	98
	SD	0.8	30.2	3.7	11.3
	n	5	5	5	5

* - Statistically different from Control $p<0.05$

Group		TBIL mg/dl	LDH U/L	CPK U/L	GGT U/L
1M CONTROL	Mean	0.1	684	921	0
	SD	0.00	223.9	1000.2	0.0
	n	5	5	5	5
2M 200 MG/M3	Mean	0.1	587	621	0
	SD	0.00	215.0	337.7	0.0
	n	5	5	5	5
3M 700 MG/M3	Mean	0.1	499	633	0
	SD	0.00	257.6	362.6	0.0
	n	5	5	5	5
4M 2000MG/M3	Mean	0.1	757	638	0
	SD	0.00	287.0	317.9	0.0
	n	5	5	5	5

Group		TPRO g/dl	ALB g/dl	CA mg/dl	PHOS mg/dl
1M CONTROL	Mean	5.5	3.1	10.8	10.7
	SD	0.18	0.11	0.22	0.86
	n	5	5	5	5
2M 200 MG/M3	Mean	5.9	3.1	11.2	11.9
	SD	0.44	0.17	0.35	0.83
	n	5	5	5	5
3M 700 MG/M3	Mean	5.7	3.1	11.2	13.2 *
	SD	0.11	0.05	0.22	1.06
	n	5	5	5	5
4M 2000MG/M3	Mean	5.5	3.0	10.8	11.3
	SD	0.08	0.04	0.26	0.54
	n	5	5	5	5

* - Statistically different from Control p<0.05

Group		CHOL mg/dl	TRIG mg/dl
1M CONTROL	Mean	52	66
	SD	4.2	26.5
	n	5	5
2M 200 MG/M3	Mean	50	58
	SD	5.7	20.6
	n	5	5
3M 700 MG/M3	Mean	49	53
	SD	3.1	27.5
	n	5	5
4M 2000MG/M3	Mean	49	48
	SD	5.4	14.1
	n	5	5

Study: 10006 Species: RAT
Time point: TIME 4

Group		BUN mg/dl	GLU mg/dl	NA mmol/L	K mmol/L
1F CONTROL	Mean	19	232	143	6.4
	SD	2.5	107.9	0.9	1.79
	n	5	5	5	5
2F 200 MG/M3	Mean	19	233	143	5.6
	SD	0.8	67.8	1.1	0.96
	n	5	5	5	5
3F 700 MG/M3	Mean	21	233	143	5.6
	SD	3.4	82.7	0.9	0.99
	n	5	5	5	5
4F 2000MG/M3	Mean	19	324	143	8.2
	SD	2.8	123.5	1.1	2.87
	n	5	5	5	5

Group		CL mmol/L	ALP U/L	ALT U/L	AST U/L
1F CONTROL	Mean	100	230	43	100
	SD	0.7	58.7	5.4	21.5
	n	5	5	5	5
2F 200 MG/M3	Mean	100	217	41	85
	SD	0.8	20.0	4.1	8.6
	n	5	5	5	5
3F 700 MG/M3	Mean	100	232	42	103
	SD	1.8	40.2	7.6	33.7
	n	5	5	5	5
4F 2000MG/M3	Mean	99	218	50	101
	SD	1.3	24.9	7.1	12.3
	n	5	5	5	5

Group		TBIL mg/dl	LDH U/L	CPK U/L	GGT U/L
1F CONTROL	Mean	0.1	523	439	0
	SD	0.00	128.8	170.6	0.4
	n	5	5	5	5
2F 200 MG/M3	Mean	0.1	440	404	0
	SD	0.00	110.5	185.3	0.0
	n	5	5	5	5
3F 700 MG/M3	Mean	0.1	456	485	1
	SD	0.00	156.4	335.2	0.5
	n	5	5	5	5
4F 2000MG/M3	Mean	0.1	406	394	1
	SD	0.00	144.3	82.1	0.5
	n	5	5	5	5

Group		TPRO g/dl	ALB g/dl	CA mg/dl	PHOS mg/dl
1F CONTROL	Mean	5.3	3.0	10.7	12.1
	SD	0.15	0.10	0.35	1.26
	n	5	5	5	5
2F 200 MG/M3	Mean	5.2	2.9	10.7	11.2
	SD	0.15	0.09	0.51	1.74
	n	5	5	5	5
3F 700 MG/M3	Mean	5.3	3.0	10.8	12.0
	SD	0.19	0.11	0.37	1.33
	n	5	5	5	5
4F 2000MG/M3	Mean	5.2	2.9	11.1	12.7
	SD	0.16	0.07	0.49	2.25
	n	5	5	5	5

Group		CHOL mg/dl	TRIG mg/dl
1F			
CONTROL	Mean	66	45
	SD	4.0	10.3
	n	5	5
2F			
200 MG/M3	Mean	71	32
	SD	5.5	13.0
	n	5	5
3F			
700 MG/M3	Mean	65	32
	SD	4.3	15.5
	n	5	5
4F			
2000MG/M3	Mean	63	44
	SD	4.5	23.7
	n	5	5

Individual Results

Group	Animal Number	COM	BUN mg/dl	GLU mg/dl	NA mmol/L
1M	701		19	190	145
CONTROL	702		18	209	143
	703	HEM	17	198	141
	704		17	236	144
	705		25	174	144
2M	706		22	216	142
200 MG/M3	707		26	256	147
	708		20	200	145
	709		19	256	143
	710		23	279	144
3M	711		19	261	143
700 MG/M3	712		19	237	143
	713		17	273	144
	714		17	188	144
	715		22	205	143
4M	716		20	203	143
2000MG/M3	717		19	145	146
	718	HEM	17	182	143
	719		22	191	143
	720		21	174	145

Group	Animal Number	K mmol/L	CL mmol/L	ALP U/L	ALT U/L
1M	701	5.0	100	281	38
CONTROL	702	6.0	100	198	43
	703	5.5	101	214	61
	704	5.8	100	277	45
	705	4.9	99	379	45
2M	706	5.9	100	233	55
200 MG/M3	707	6.1	101	263	43
	708	5.9	101	217	47
	709	6.5	97	243	39
	710	6.0	97	349	52
3M	711	6.6	99	240	68
700 MG/M3	712	6.5	97	259	48
	713	7.6	99	190	39
	714	5.7	100	261	49
	715	5.6	98	298	49
4M	716	5.5	99	300	42
2000MG/M3	717	4.4	101	284	41
	718	5.3	100	225	47
	719	5.4	100	294	44
	720	4.4	99	265	37

Group	Animal Number	AST U/L	TBIL mg/dl	LDH U/L	CPK U/L
1M CONTROL	701	84	0.1	760	507
	702	101	0.1	766	500
	703	181	0.1	941	2704
	704	103	0.1	608	555
	705	89	0.1	343	338
2M 200 MG/M3	706	116	0.1	751	1168
	707	116	0.1	658	601
	708	105	0.1	698	561
	709	99	0.1	615	533
	710	85	0.1	213	240
3M 700 MG/M3	711	165	0.1	925	1229
	712	93	0.1	407	543
	713	74	0.1	237	304
	714	86	0.1	507	406
	715	107	0.1	417	685
4M 2000MG/M3	716	96	0.1	984	504
	717	80	0.1	538	272
	718	107	0.1	604	1122
	719	97	0.1	1143	551
	720	108	0.1	517	741

Group	Animal Number	GGT U/L	TPRO g/dl	ALB g/dl	CA mg/dl
1M CONTROL	701	0	5.7	3.2	10.8
	702	0	5.7	3.2	10.9
	703	0	5.3	3.0	10.5
	704	0	5.4	3.0	10.8
	705	0	5.5	3.0	11.1
2M 200 MG/M3	706	0	5.6	3.0	10.8
	707	0	6.6	3.4	11.7
	708	0	5.9	3.2	10.9
	709	0	5.7	3.1	11.2
	710	0	5.5	3.0	11.2
3M 700 MG/M3	711	0	5.7	3.0	11.2
	712	0	5.5	3.0	11.2
	713	0	5.8	3.1	11.5
	714	0	5.7	3.1	10.9
	715	0	5.6	3.1	11.1
4M 2000MG/M3	716	0	5.6	3.0	10.6
	717	0	5.6	3.1	11.0
	718	0	5.5	3.0	11.1
	719	0	5.4	3.0	10.5
	720	0	5.5	3.0	10.7

Group	Animal Number	PHOS mg/dl	CHOL mg/dl	TRIG mg/dl
1M	701	9.8	47	78
CONTROL	702	12.0	58	36
	703	10.3	52	46
	704	11.2	49	66
	705	10.4	52	103
2M	706	11.2	45	79
200 MG/M3	707	10.9	58	35
	708	12.1	44	49
	709	12.9	50	47
	710	12.4	52	81
3M	711	12.8	52	47
700 MG/M3	712	13.7	53	33
	713	14.8	48	24
	714	12.5	47	68
	715	12.2	46	92
4M	716	10.7	49	46
2000MG/M3	717	11.5	46	34
	718	11.5	58	40
	719	12.0	44	49
	720	10.8	47	71

Group	Animal Number	BUN mg/dl	GLU mg/dl	NA mmol/L	K mmol/L
1F	801	22	179	144	5.8
CONTROL	802	17	424	144	8.4
	803	19	174	142	8.1
	804	16	182	144	5.0
	805	21	200	143	4.5
2F	806	19	210	144	5.6
200 MG/M3	807	20	339	143	7.0
	808	18	189	145	4.6
	809	18	257	142	5.9
	810	19	169	143	4.8
3F	811	26	212	144	6.4
700 MG/M3	812	22	368	144	6.8
	813	17	205	144	5.1
	814	19	235	142	5.2
	815	21	144	143	4.4
4F	816	18	479	142	8.5
2000MG/M3	817	22	413	141	12.5
	818	16	273	143	7.0
	819	16	165	144	4.6
	820	21	290	143	8.4

Group	Animal Number	CL mmol/L	ALP U/L	ALT U/L	AST U/L
1F CONTROL	801	100	260	44	126
	802	99	253	42	83
	803	100	172	51	121
	804	101	166	36	89
	805	100	300	41	82
2F 200 MG/M3	806	101	216	46	98
	807	99	227	37	90
	808	101	203	37	77
	809	100	193	44	80
	810	100	244	42	81
3F 700 MG/M3	811	101	229	43	106
	812	97	261	54	156
	813	101	185	43	103
	814	101	202	35	70
	815	101	282	36	78
4F 2000MG/M3	816	98	214	56	102
	817	97	257	59	112
	818	100	195	48	105
	819	100	199	46	106
	820	99	225	42	80

Group	Animal Number	TBIL mg/dl	LDH U/L	CPK U/L	GGT U/L
1F CONTROL	801	0.1	478	665	0
	802	0.1	354	237	0
	803	0.1	609	447	0
	804	0.1	687	532	0
	805	0.1	485	313	1
2F 200 MG/M3	806	0.1	456	426	0
	807	0.1	277	717	0
	808	0.1	411	263	0
	809	0.1	472	325	0
	810	0.1	582	291	0
3F 700 MG/M3	811	0.1	309	460	0
	812	0.1	577	1049	1
	813	0.1	634	440	0
	814	0.1	284	179	1
	815	0.1	475	298	1
4F 2000MG/M3	816	0.1	375	392	0
	817	0.1	274	353	1
	818	0.1	489	459	1
	819	0.1	612	485	0
	820	0.1	281	281	1

Group	Animal Number	TPRO g/dl	ALB g/dl	CA mg/dl	PHOS mg/dl
1F CONTROL	801	5.1	2.9	10.7	11.9
	802	5.5	3.1	11.3	13.4
	803	5.4	3.1	10.7	13.4
	804	5.3	2.9	10.5	11.5
	805	5.3	3.0	10.4	10.5
2F 200 MG/M3	806	5.2	2.9	10.8	11.9
	807	5.2	3.0	11.5	13.8
	808	5.1	3.0	10.4	9.8
	809	5.0	2.8	10.2	11.0
	810	5.4	3.0	10.5	9.5
3F 700 MG/M3	811	5.2	2.9	11.0	12.8
	812	5.2	2.9	11.3	13.6
	813	5.3	3.1	10.4	10.9
	814	5.1	2.9	10.5	12.4
	815	5.6	3.1	10.9	10.4
4F 2000MG/M3	816	5.1	2.8	11.6	13.4
	817	5.3	2.9	11.4	16.3
	818	5.0	2.9	10.6	11.3
	819	5.4	3.0	10.5	10.8
	820	5.3	2.9	11.2	11.6

Group	Animal Number	CHOL mg/dl	TRIG mg/dl
1F CONTROL	801	63	50
	802	72	55
	803	62	40
	804	68	30
	805	67	52
2F 200 MG/M3	806	69	30
	807	70	37
	808	80	22
	809	65	20
	810	70	52
3F 700 MG/M3	811	66	24
	812	65	37
	813	64	23
	814	58	19
	815	70	57
4F 2000MG/M3	816	60	64
	817	66	75
	818	57	25
	819	68	24
	820	64	33

APPENDIX G. INDIVIDUAL ANIMAL ORGAN WEIGHTS

Males			Individual Organ Weights (g)			
Timepoint	Dose Group	Animal Number	Adrenals	Right Kidney	Left Kidney	Liver
6 hr	0	101	0.032	0.70	0.69	7.32
		102	0.040	0.66	0.66	7.27
		103	0.037	0.60	0.60	6.96
		104	0.040	0.63	0.63	7.27
		105	0.044	0.65	0.60	7.06
	200	106	0.030	0.63	0.63	6.83
		107	0.032	0.62	0.60	7.51
		108	0.033	0.63	0.65	7.10
		109	0.033	0.59	0.63	7.39
		110	0.033	0.61	0.60	7.22
	700	111	0.041	0.67	0.69	7.46
		112	0.035	0.55	0.59	7.16
		113	0.041	0.62	0.61	7.00
		114	0.032	0.63	0.63	7.36
		115	0.036	0.56	0.54	6.96
	2000	116	0.032	0.57	0.56	6.71
		117	0.041	0.60	0.62	7.48
		118	0.037	0.60	0.59	6.60
		119	0.038	0.54	0.63	7.23
		120	0.043	0.55	0.58	6.31
6 hr + 11 days	0	301	0.042	0.79	0.79	8.60
		302	0.040	0.66	0.67	8.02
		303	0.045	0.72	0.77	8.38
		304	0.050	0.76	0.79	8.11
		305	0.030	0.68	0.73	7.46
	200	306	0.035	0.70	0.73	8.42
		307	0.050	0.75	0.79	8.03
		308	0.043	0.79	0.79	8.94
		309	0.038	0.74	0.81	8.59
		310	0.049	0.77	0.79	8.97
	700	311	0.039	0.74	0.72	7.92
		312	0.043	0.68	0.67	7.62
		313	0.037	0.66	0.68	7.49
		314	0.056	0.85	0.81	8.84
		315	0.041	0.74	0.79	8.92
	2000	316	0.039	0.71	0.71	7.85
		317	0.035	0.67	0.70	7.88
		318	0.048	0.65	0.65	7.02
		319	0.041	0.69	0.67	7.62
		320	0.031	0.64	0.65	7.49

Males			Individual Organ Weights (g)			
Timepoint	Dose Group	Animal Number	Adrenals	Right Kidney	Left Kidney	Liver
5 Days	0	501	0.048	0.62	0.63	7.11
		502	0.037	0.64	0.64	7.69
		503	0.037	0.60	0.58	6.81
		504	0.049	0.66	0.65	7.28
		505	0.037	0.64	0.63	6.91
	200	506	0.040	0.71	0.72	8.83
		507	0.048	0.58	0.60	7.63
		508	0.037	0.66	0.68	7.69
		509	0.049	0.74	0.77	9.42
		510	0.050	0.65	0.66	6.96
	700	511	0.035	0.64	0.68	7.41
		512	0.035	0.68	0.70	8.18
		513	0.045	0.66	0.68	8.09
		514	0.039	0.64	0.66	7.56
		515	0.037	0.70	0.74	8.23
	2000	516	0.037	0.68	0.77	7.84
		517	0.027	0.58	0.62	7.06
		518	0.051	0.66	0.70	7.91
		519	0.048	0.67	0.71	7.63
		520	0.035	0.57	0.59	6.58
10 Days	0	701	0.047	0.72	0.78	7.57
		702	0.041	0.61	0.60	5.92
		703	0.049	0.58	0.60	5.68
		704	0.050	0.64	0.67	6.89
		705	0.018*	0.63	0.67	7.57
	200	706	0.052	0.74	0.72	6.67
		707	0.069	0.76	0.71	6.98
		708	0.046	0.74	0.74	7.43
		709	0.052	0.70	0.69	7.05
		710	0.050	0.76	0.75	8.76
	700	711	0.046	0.77	0.72	8.25
		712	0.046	0.67	0.65	6.62
		713	0.042	0.76	0.77	6.55
		714	0.049	0.79	0.79	8.41
		715	0.042	0.70	0.78	8.77
	2000	716	0.039	0.71	0.71	7.68
		717	0.051	0.77	0.76	7.42
		718	0.041	0.73	0.68	6.59
		719	0.041	0.71	0.70	7.65
		720	0.049	0.74	0.74	8.08
* Right adrenal missing, not recovered at necropsy						

Females			Individual Organ Weights (g)			
Timepoint	Dose Group	Animal Number	Adrenals	Right Kidney	Left Kidney	Liver
6 hr	0	201	0.028	0.52	0.51	5.32
		202	0.034	0.50	0.49	4.86
		203	0.045	0.53	0.52	4.94
		204	0.042	0.52	0.55	4.71
		205	0.050	0.52	0.53	5.12
	200	206	0.046	0.50	0.49	5.15
		207	0.058	0.53	0.52	5.04
		208	0.044	0.46	0.46	4.56
		209	0.044	0.47	0.49	4.35
		210	0.039	0.41	0.41	4.17
	700	211	0.039	0.49	0.46	4.72
		212	0.036	0.50	0.49	5.01
		213	0.041	0.50	0.49	4.78
		214	0.039	0.46	0.49	4.63
		215	0.046	0.47	0.46	4.59
	2000	216	0.040	0.48	0.48	4.94
		217	0.042	0.51	0.44	4.64
		218	0.048	0.55	0.55	5.23
		219	0.052	0.51	0.47	4.79
		220	0.052	0.54	0.55	5.32
6 hr + 11 days	0	401	0.041	0.54	0.58	5.84
		402	0.040	0.52	0.54	5.56
		403	0.053	0.57	0.53	5.39
		404	0.054	0.60	0.57	6.03
		405	0.047	0.57	0.56	5.49
	200	406	0.051	0.59	0.60	5.73
		407	0.049	0.60	0.57	5.21
		408	0.044	0.57	0.61	5.59
		409	0.059	0.52	0.53	5.54
		410	0.042	0.51	0.54	5.49
	700	411	0.052	0.51	0.56	5.83
		412	0.061	0.63	0.63	6.03
		413	0.051	0.54	0.54	5.32
		414	0.055	0.63	0.63	6.26
		415	0.054	0.55	0.58	5.72
	2000	416	0.046	0.55	0.54	5.63
		417	0.046	0.54	0.53	5.90
		418	0.046	0.51	0.53	5.38
		419	0.058	0.60	0.58	5.86
		420	0.059	0.52	0.54	5.32

Females			Individual Organ Weights (g)			
Timepoint	Dose Group	Animal Number	Adrenals	Right Kidney	Left Kidney	Liver
5 Days	0	601	0.043	0.46	0.54	5.52
		602	0.052	0.51	0.52	5.77
		603	0.050	0.51	0.50	5.12
		604	0.057	0.51	0.53	5.30
		605	0.072	0.56	0.56	5.90
	200	606	0.054	0.57	0.57	5.98
		607	0.051	0.57	0.58	4.97
		608	0.058	0.57	0.57	5.73
		609	0.048	0.50	0.53	5.42
		610	0.047	0.58	0.51	4.61
	700	611	0.052	0.50	0.55	5.38
		612	0.047	0.49	0.45	4.89
		613	0.051	0.52	0.52	5.09
		614	0.045	0.44	0.45	4.56
		615	0.054	0.53	0.54	5.46
	2000	616	0.044	0.52	0.54	5.34
		617	0.044	0.49	0.49	4.59
		618	0.055	0.54	0.51	5.79
		619	0.056	0.54	0.54	5.01
		620	0.052	0.53	0.52	5.23
10 Days	0	801	0.054	0.58	0.57	5.64
		802	0.056	0.60	0.57	5.81
		803	0.056	0.56	0.53	4.95
		804	0.050	0.52	0.53	4.87
		805	0.043	0.48	0.44	4.63
	200	806	0.050	0.54	0.55	5.10
		807	0.052	0.53	0.55	4.98
		808	0.037	0.50	0.47	4.42
		809	0.053	0.51	0.51	4.59
		810	0.040	0.50	0.51	5.37
	700	811	0.042	0.47	0.48	5.12
		812	0.041	0.53	0.53	5.23
		813	0.060	0.50	0.49	4.39
		814	0.047	0.56	0.55	4.88
		815	0.045	0.52	0.57	5.62
	2000	816	0.063	0.60	0.59	5.84
		817	0.043	0.56	0.56	5.34
		818	0.057	0.51	0.54	5.15
		819	0.054	0.52	0.55	4.89
		820	0.045	0.58	0.57	5.16

APPENDIX H. PATHOLOGY TABLES

NASAL AIRWAYS BY LEVEL

Squamous Epithelium Inflammation – Nose Tip Level

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	2 (0.4)	1 (0.2)	5 (1)	2 (0.4)
200	3 (0.8)	3 (0.6)	3 (0.6)	3 (0.6)
700	3 (0.8)	3 (0.6)	5 (1)	5 (1)
2000	5 (1)	5 (1)	5 (1)	3 (0.6)
Females				
0	2 (0.4)	0 (0)	0 (0)	1 (0.2)
200	4 (0.8)	0 (0)	1 (0.2)	0 (0)
700	0 (0)	0 (0)	1 (0.2)	3 (0.6)
2000	2 (0.4)	0 (0)	3 (0.6)	4 (0.8)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Squamous Epithelium Hyperplasia – Nose Tip Level

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	1 (0.2)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	2 (0.4)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	1 (0.2)	0 (0)	4 (1.0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Transitional/Respiratory Epithelium Inflammation – Nose Level I

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	3 (0.8)	1 (0.2)	0 (0)	0 (0)
200	3 (0.8)	0 (0)	2 (0.4)	1 (0.2)
700	4 (1)	2 (0.4)	0 (0)	0 (0)
2000	5 (1)	5 (1)	0 (0)	1 (0.2)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	2 (0.4)	0 (0)	0 (0)	0 (0)
700	4 (0.8)	0 (0)	1 (0.2)	0 (0)
2000	1 (0.2)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Transitional/Respiratory Epithelium Hyperplasia – Nose Level I

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	1 (0.2)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	1 (0.2)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Transitional/Respiratory Epithelium Inflammation – Nose Level II

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	1 (0.4)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Nasolacrimal Duct Goblet Cell Hypertrophy/Hyperplasia – Nose Level V

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	4 (0.8)	5 (1)
2000	0 (0)	0 (0)	5 (1)	5 (1)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	5 (1)	5 (1)
2000	0 (0)	0 (0)	5 (1)	5 (1)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

TRACHEA

Trachea Abnormalities Detected

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

LARYNX

Larynx Abnormalities Detected

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

LUNGS

Inflammation - Focal, Chronic

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	4 (0.8)	1 (0.2)	2 (0.4)	2 (0.4)
200	3 (0.6)	3 (0.6)	2 (0.4)	0 (0)
700	2 (0.4)	2 (0.4)	2 (0.4)	2 (0.4)
2000	1 (0.2)	2 (0.4)	0 (0)	0 (0)
Females				
0	3 (0.6)	2 (0.4)	1 (0.2)	2 (0.4)
200	4 (0.8)	3 (0.6)	2 (0.4)	0 (0)
700	0 (0)	1 (0.2)	3 (0.6)	3 (0.6)
2000	0 (0)	3 (0.6)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

LIVER

Inflammation - Focal, Chronic

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	1 (0.2)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	3 (0.6)	1 (0.2)
700	0 (0)	0 (0)	2 (0.4)	0 (0)
2000	0 (0)	0 (0)	2 (0.4)	0 (0)
Females				
0	1 (0.2)	2 (0.4)	0 (0)	0 (0)
200	1 (0.2)	1 (0.2)	1 (0.2)	0 (0)
700	1 (0.2)	0 (0)	1 (0.2)	0 (0)
2000	0 (0)	1 (0.2)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Cyst

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	1 (0.2)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Hepatodiaphragmatic Nodule

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	1 (0.2)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	1 (0.2)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	1 (P)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	1 (P)

Notes: n = 5/group. P = Finding present, severity not scored. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

KIDNEYS

Regeneration (Tubular Epithelium)

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	1 (0.2)	0 (0)	0 (0)	0 (0)
200	0 (0)	1 (0.2)	0 (0)	1 (0.2)
700	1 (0.2)	0 (0)	0 (0)	1 (0.2)
2000	0 (0)	1 (0.2)	0 (0)	0 (0)
Females				
0	2 (0.4)	1 (0.2)	0 (0)	0 (0)
200	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)
700	1 (0.2)	1 (0.2)	0 (0)	0 (0)
2000	1 (0.2)	1 (0.2)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

Infarct

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	1 (0.2)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

SPLEEN

Spleen Abnormalities Detected

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

ADRENAL GLANDS

Adrenal Gland Abnormalities Detected

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

HEART

Cardiomyopathy

Dose Group (mg/m^3)	Acute Exposure Group	Acute + Recovery Exposure Group	Five-Day Exposure Group	Ten-Day Exposure Group
Males				
0	0 (0)	0 (0)	0 (0)	1 (0.2)
200	0 (0)	0 (0)	0 (0)	0 (0)
700	0 (0)	0 (0)	0 (0)	1 (0.2)
2000	0 (0)	2 (0.4)	0 (0)	0 (0)
Females				
0	0 (0)	0 (0)	0 (0)	1 (0.2)
200	0 (0)	1 (0.2)	0 (0)	0 (0)
700	0 (0)	1 (0.2)	0 (0)	0 (0)
2000	0 (0)	0 (0)	0 (0)	0 (0)

Notes: n = 5/group. Data expressed as incidence (average severity grade) where severity is measured using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high)

MACRO/MICRO CORRELATION TABLE

Animal Number	Exposure Conc. (mg/m^3)	Duration	Sex	Necropsy Observation	Corresponding Microscopic Finding
110	200	A	M	Median lobe-diaphramtic nodule 5 mm in dia	Hepatodiaphragmatic nodule
405	0	A+R	F	3x6x8 mm diaphragmatic hernia	Hepatodiaphragmatic nodule
616	2000	5	F	LT-Focus, 1 mm dia, white	Alveolus inflammation, grade 3
810	200	10	F	Median Lobe-HDN, 5 mm dia	Hepatodiaphragmatic nodule
817	2000	10	F	Median Lobe-HDN, 2 mm dia	Hepatodiaphragmatic nodule

Notes: 5 = five-day exposure; 10 = ten-day exposure; A = acute; A+R = acute + recovery; dia = diameter; F = female; M = male; neg = negative

GMS and Gram Stains of Lungs

Animal Number	Exposure Concentration (mg/m^3)	Duration	Sex	GMS Stain	Gram Positive Organisms	Gram Positive Organisms
101	0	A	M	neg	neg	neg
205	0	A	F	neg	neg	neg
403	0	A+R	F	neg	neg	neg
605	0	5	F	neg	neg	neg
701	0	10	M	neg	neg	neg
115	700	A	M	neg	neg	neg
515	700	5	M	neg	neg	neg
615	2000	5	F	neg	neg	neg
714	700	10	M	neg	neg	neg
815	700	10	F	neg	neg	neg

Notes: 5 = five-day exposure; 10 = ten-day exposure; A = acute; A+R = acute + recovery; F = female; M = male; neg = negative

APPENDIX I. CYTOKINES PCR ANALYSIS SUMMARY

Data source - (**http://www.sabiosciences.com/rt_pcr_product/HTML/PARN-011A.html**): SABioscience's "Inflammatory Cytokines & Receptors PCR Array", Rat array PARN-011E-4 (96 genes x 4 samples, 384 well array plate). This array contains:

Chemokine Genes: RGD1561905 (C5), Ccl11, Ccl12, Ccl17, Ccl19, Ccl2, Ccl20, Ccl21b, Ccl22, Ccl24, Ccl25, Ccl3, Ccl4, Ccl5, Ccl6, Ccl7, Ccl9, Cx3cl1, Cxcl1, Cxcl10, Cxcl11, Cxcl12 (Sdf1), Cxcl2, Cxcl5, Cxcl9, Il13, Cxcl4 (Pf4)

Chemokine Receptors: Ccr1, Ccr2, Ccr3, Ccr4, Ccr5, Ccr6, Ccr7, Ccr8, Ccr9, Cx3cr1, Cxcr3, Gpr2, Il8ra, Il8rb, Xcr1

Cytokine Genes: Ifng (IFNγ), Il10, Il11, Il13, Il15, Il16, Il17b, Il18, Il1a, Il1b, Il1f5, Il1f6, Il3, Il4, Il5, Itgam, Itgb2, Lta, Ltb, Mif, Scye1, Spp1, Tgfb1, Tnf, Cd40lg (Tnfsf5)

Cytokine Receptors: Ifng (IFNγ), Il10ra, Il13, Il13ra1, Il1r1, Il1r2, Il2rb, Il2rg, Il5ra, Il6ra, Il6st, Tnfrsf1a (TNFR1), Tnfrsf1b (TNFR2)

Other Genes Involved in the Inflammatory Response: Abcf1, Bcl6, Blr1, C3, Casp1, Crp, Il1r1, Il8rb, Tollip

(Plus control genes for Rat Genomic DNA contamination, Reverse Transcriptase and positive PCR controls)

Data was analyzed with SABioscience's online RT^2 Profiler PCR Array Data Analysis web site http://pcrdataanalysis.sabiosciences.com/pcr/arrayanalysis.php using the provider's recommended default settings

Dose response

Samples were pooled by inhalation exposure dose
Control group = all males and females, all time points, 0 mg/m^3 (40 samples total)
Group 1 = all males and females, all time points, 200 mg/m^3 (40 samples total)
Group 2 = all males and females, all time points, 700 mg/m^3 (40 samples total)
Group 3 = all males and females, all time points, 2000 mg/m^3 (40 samples total)

Gender response

Pooled samples across all time points as above, but male and female groups analyzed separately (20 samples per dose).

SUMMARY OF RESULTS

There were only minimal changes in gene expression at the lowest treatment dose of 200mg/m^3. The predominant changes in gene expression occurred at the highest treatment dose of 2000 mg/m^3 and mainly involved Chemokine genes. There were little differences between sexes at the treatment doses, and these in turn tended to mirror the results from the comparisons strictly by dose (that is, both sexes showed similar dose response effects, both in magnitude and direction of changes in gene expression).

Dose response

A single gene (Il1f6 = Interleukin 1 family member 6) was significant by Student's t-test ($p < 0.05$) at the lowest treatment dose of 200 mg/m^3. At 700 mg/m^3 there were 32 genes with significant differential expression and 53 at 2000 mg/m^3, with 29 of those being common to both concentrations. The magnitude of changes in gene expression at the 200 and 700 mg/m3 doses was low however, with only a single gene (Spp1 = Secreted Phosphoprotein 1) showing a fold change greater than 2 (700 mg/m^3). There were 13 genes at 2000 mg/m^3 that had a fold change of greater than 2 (all with p-values much less than 0.05), and all but one of these was a Chemokine Gene (one, Ccr8 was a Chemokine Receptor).

Gender differences

While there were differences in significance and expression level of some genes between sexes, there seems little biological significance to these. In both sexes, the predominant effect on gene expression was the effect of dose. Most change in gene expression was again seen at the highest treatment dose (2000 mg/m^3) and was predominantly amongst the Chemokine genes. These also tended to be the same genes significant within this class as seen in the combined-gender dose response analysis. Gender differences appear to be limited to relatively subtle differences in the expression intensity of significant genes.

APPENDIX J. SENSORY IRRITATION DATA

Animal Weights on 06/28/2010

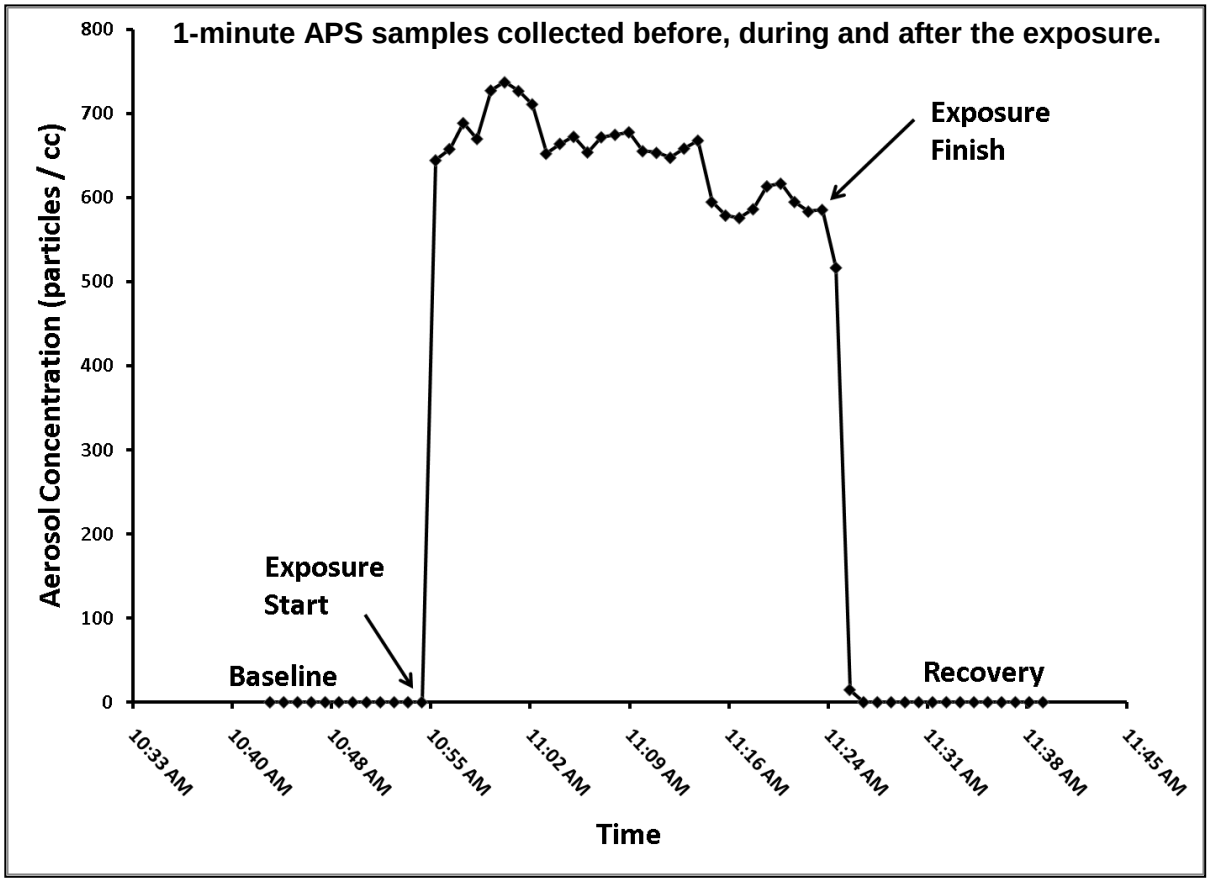
Animal Number (10006 -)	Weight (grams)
901	31.0
902	27.5
903	29.5
904	26.1

Miran Aerosol/Vapor Concentrations

Miran Sample Number	Sample Time	Aerosol/Vapor Concentration (mg/m^3)
1	11:02 AM	1807.5
2	11:03 AM	1832.1
3	11:05 AM	1848.6
4	11:07 AM	1889.8
5	11:10 AM	1923.1
6	11:12 AM	1931.4
7	11:14 AM	1948.1
8	11:16 AM	1948.1
9	11:18 AM	1973.2
10	11:20 AM	1948.1
11	11:22 AM	1973.2
12	11:24 AM	1973.2
	Mean	1916.4
	Standard Deviation	58.2
	Coefficient of Variation (%)	3.0
	- Aerosol Concentration – Mass Weight Filter (mg/m^3)	407.0
	Percentage of Aerosol (%)	21.2

Mass Median Aerodynamic Particle Size

Aerodynamic Particle Sizer Samples	Diameter (µm)	Geometric Standard Deviation
Average	1.46	1.44
Maximum	1.70	1.53
Minimum	1.42	1.43
Standard Deviation	0.06	0.02
Number of Samples	29	29
Sample time (seconds)	60	60

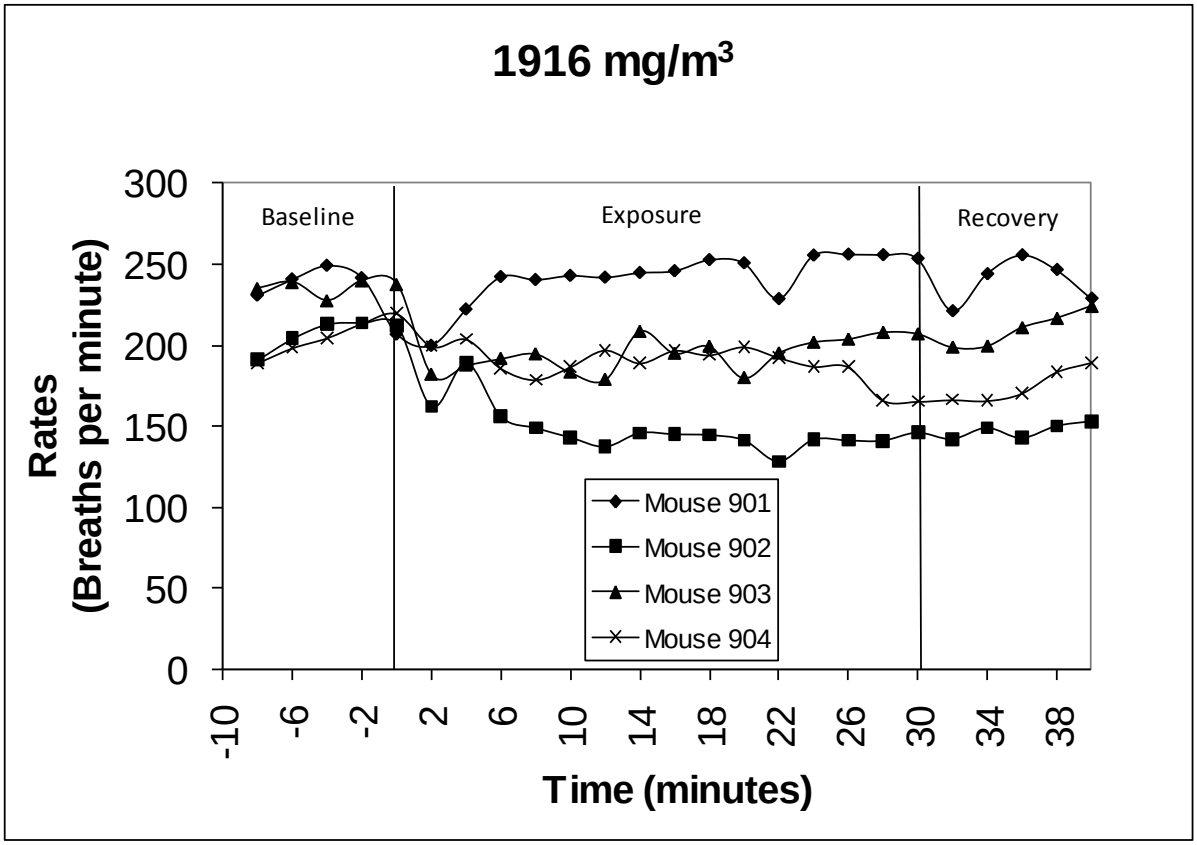


Particle Counts capture by an Aerodynamic Particle Sizer.

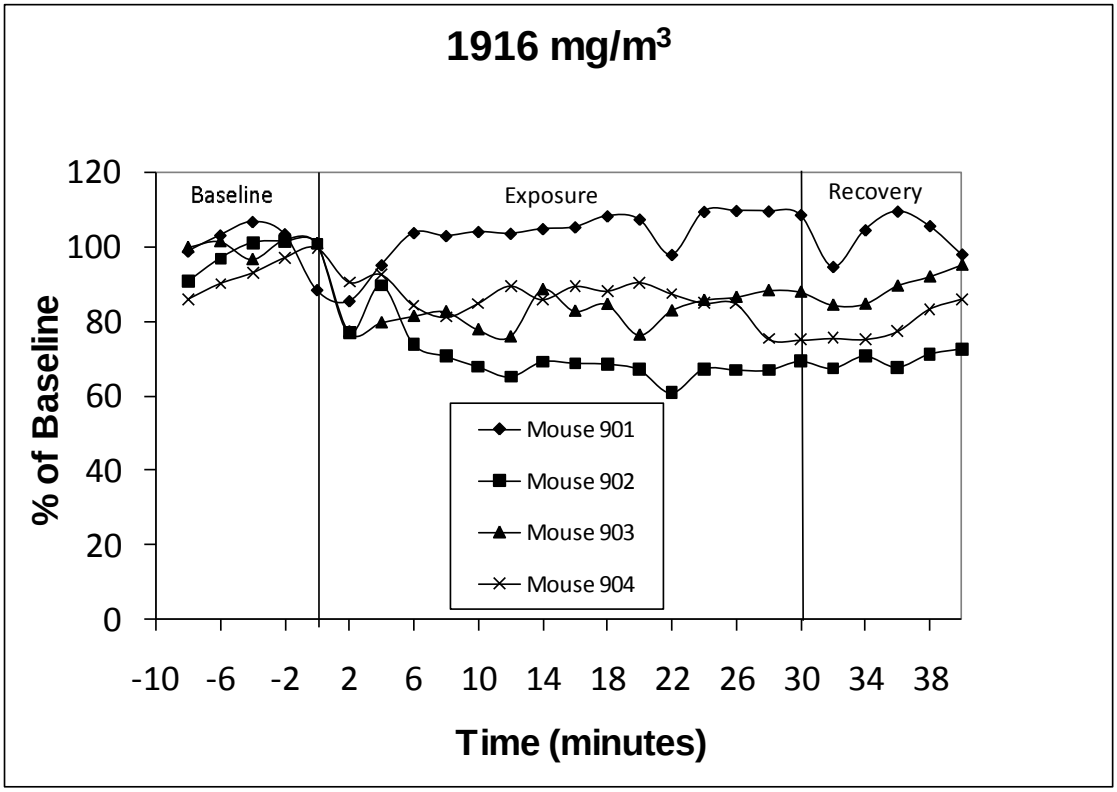
Summary of Animal Response Data

Concentration (mg/m^3)	Animal Number	Body Weight (g)	% Respiratory Decrease	% Recovery	Irritation Type**	Gross Observations*
	901	31.0	-5.2	125.4	Sensory/Slight	NOA
1916.4	902	27.5	-34.6	75.5	Sensory/Moderate	NOA
	903	29.5	-26.2	96.2	Sensory/Moderate	NOA
	904	26.1	-25.2	87.2	Sensory/Moderate	NOA
	Mean	28.5	-22.8	96.1		
	S.D.	2.2	12.5	21.3		

Notes: *NOA – no observable abnormalities seen before, during or after exposures; **severity categorized as slight = 12-19%; moderate = 20-49%; extreme ≥ 50%



Individual Respiratory Rates for the 1916 mg/m^3 HEFA-F Exposure



Individual Respiratory Rates as a Percentage of the Baseline

LIST OF ACRONYMS

ANOVA	analysis of variance
APS	aerodynamic particle sizer
DTIC	Defense Technical Information Center
EPL	Experimental Pathology Laboratories, Inc.
GMS	Gomori methenamine silver
GSD	geometric standard deviation
HEFA-F	hydroprocessed esters and fatty acids - mixed fats
HEPA	high efficiency particulate air
HRJ	hydro-treated renewable jet
IR	infrared
MCP-1	monocyte chemoattractant protein 1
MMAD	mass median aerodynamic diameter
MWF	mass weight (gravimetric) filter
NAD	no abnormalities detected
NBF	neutral buffered formalin
OD	outer diameter
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
RTP	Research Triangle Park
SD	standard deviation
SPK	synthetic paraffinic kerosene
Spp1	Secreted Phosphoprotein 1
TP	total port
U.S. EPA	U.S. Environmental Protection Agency